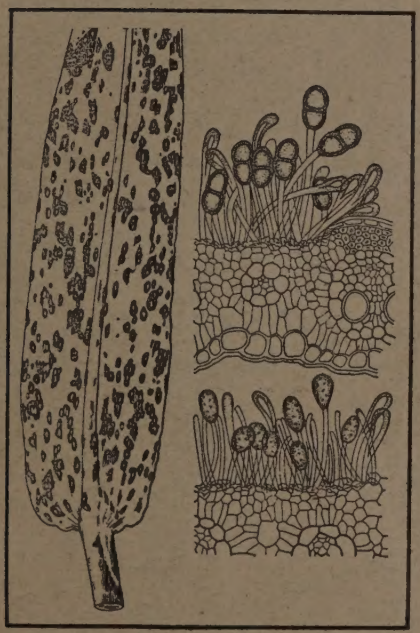
INDIAN PHYTOPATHOLOGY

VOLUME III 1950 NUMBER 2



PUBLISHED FOR
INDIAN PHYTOPATHOLOGICAL SOCIETY
PUSA BUILDINGS, NEW DELHI 12
INDIA

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PRINCIPAL DISEASES AND DECAYS OF OAKS IN INDIA

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(Accepted for publication November 13, 1950)

SPECIES of *Quercus* are of great importance in forestry in Europe, United States and elsewhere. Oak timber enjoys a reputation for its strength and natural durability. Indian oaks are present only in the Himalayas and though not comparable in quality with the better grade oaks obtained elsewhere, they are important economically and silviculturally. They yield excellent firewood and charcoal and constitute an important association with the more valuable conifers with which they grow. Locally, oak timber is used for building and constructional purposes. The present paper deals with the principal diseases and decays of three important species of *Quercus* namely *Q. incana*, *Q. dilatata* and *Q. semicarpifolia* which grow in the Western Himalayas. Some of the pathogens are recorded in Europe and America as fungi of major importance. In India, they have so far been casually recorded. An attempt is, therefore, made to give an account of these fungi as they occur in our country.

Attempts have been made to study the anatomical characters of the sporophores as well as characters of the fungi in culture, which are recognised as valuable aids in the identification of wood-rotting fungi. Gross characters of decay have been described which also help in the diagnosis of the organism causing the rot. Fresh fruit-bodies were incubated under moist conditions when the hymenial layer usually revived. At this stage, spore prints were taken and sections were made to observe basidia. Measurements were taken under magnification of about 1,000 times.

Cultural characters of the fungi have been studied on 2% malt agar in Petri dishes kept in the dark at 24°C. Ridgway's (1912) Color Standards and Color Nomenclature has been used in describing the colours in general. Oxidase tests as described by Bavendamm (1928) were made by growing fungi on malt agar containing 0.5 per cent gallic acid or 0.3 per cent tannic acid. Fungi causing white rot usually form dark diffusion zones underneath the fungal mat on such media while those causing brown rot do not give any such reaction. The white rotters decolourise or bleach the violet colour of malt agar containing 0.007 per cent gentian violet which the brown rotters do not (Preston and Mclennan, 1948). This test was also carried out to distinguish white rot fungi from brown rot fungi.

Descriptions of the Causal Fungi and the Rots

Stereum princeps Jungh.

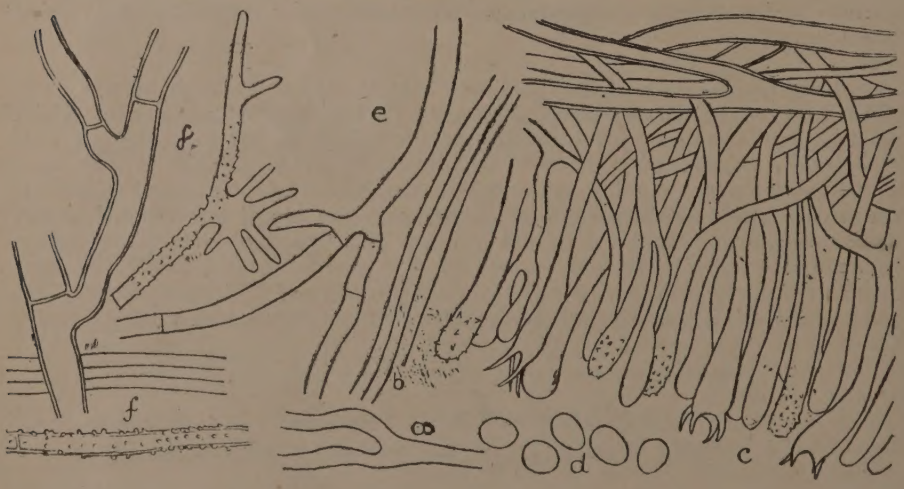
(Pl. I, figs. 1, 8 and 9; Pl. II, figs. 10 and 11 and text-fig. 1)

Stereum princeps and *Stereum scytale* Berk. are regarded as synonymous. Berkeley remarked (Massee, 1891) that thick specimens of *S. scytale* approach the magnificent *S. princeps*. Bresadola was of opinion that *S. scytale* is identical with *S. princeps* and Miss Wakefield follows this view in her determination, using the

name *S. princeps* as being the earlier. In response to a request for determining the identity of *S. princeps* and *S. scytale*, Miss Wakefield kindly re-examined the type specimen of *S. scytale*, the Bresadola specimen of *S. princeps* and an Indian specimen of *S. princeps* and confirmed that there is no specific difference between them.

OCCURRENCE. *Stereum princeps* is a tropical fungus and has been recorded in Java (type locality) and Burma. In India, the fungus is very common in the Western Himalayas up to an altitude of about 8,000 ft. and occurs in the Khasi hills and the Eastern Himalayas. It is also found, though rarely, in the plains of N. India. The fungus is a common saprophyte on oak stumps and logs but is also a wound parasite on various species of *Quercus*. Fruit-bodies of the fungus develop on fire-scars, wounds, cankers, etc., on living trees (Pl. II, fig. 10).

SPOROPHORE. Fruit-bodies sessile, reflexed, attached by broad base, usually gregarious, imbricate, large, about 1-1.4 mm. thick, divided into irregular lobes, coriaceous when fresh, hard, brittle and finely cracking when dry. Upper surface glabrous, concentrically zonate (Pl. I, fig. 8), brown, sepia to black. Context zoned (usually 3-4) with varying shades of brown and sepia ; hyphae longitudinally arranged and are thin-walled, hyaline, closely septate, branched (Text-fig. 1,a) 1.4-2.9 μ broad and thick-walled (Text-fig. 1,b), brown in mass but light brown to nearly hyaline individually, 2.8-4.2 μ broad. Hymenium faintly zoned, uneven due to raised pustules (Pl.I, fig. 9), 'pale ochraceous buff'. Basidia (Text-fig. 1,c) cylindric, 7-11.5 $\times$2.8-4.3 μ with 4 sterigmata on each, latter up to 3.5 μ long, 1 μ broad at base. Spores hyaline, round to oval, thin-walled (Text-fig. 1,d), 3.2-5.4 $\times$2.5-5 μ, average 4.5 $\times$ 3.3 μ. Paraphyses with fine projections on the walls (bottle-brush type), numerous in the hymenium (Text-fig. 1,c).



Text-fig. 1. *Stereum princeps*, a, thin-walled hypha from context ; b, thick-walled hypha from context ; c, hymenial layer showing basidia and bottle-brush paraphyses ; d, basidiospores ; e, thin-walled hypha in culture ; f, thick-walled hyphae in culture. All $\times$ 1300,

THE ROT. The incipient stage appears as a reddish brown discolouration in the wood in which pockets develop later. The pockets are elongate and the wood inside them is at first reddish brown but with age is turned white and fibrous (Pl. II, fig. 11).

FUNGUS IN CULTURE (context culture from sporophore on oak).

(a) Growth characters. Growth* slow, 6-8 mm. Mat felty (Pl. I, fig. 1), white but soon developing 'orange' and 'capucine yellow' which deepen to 'ochraceous orange' with age. Undersurface 'orange-buff' turning 'amber brown' later. On gallic and tannic acid agars, the diffusion zones weak, growth about 5 mm. On Gentian violet agar, growth slow, medium slightly discoloured.

(b) Hyphal characters. *Aerial mycelium* : (a) hyphae hyaline, thin-walled, much branched with simple septa (Text-fig. 1,e), 2.5-4.2 μ broad ; (b) hyphae yellow-brown, thick-walled with lumen large or small, much branched with frequent swellings bearing fine projections or with minute projections all over hyphae or projections lacking (Text-fig. 1,f) 1.4-5.7 μ broad. *Submerged mycelium* : Hyaline, thin-walled, much branched hyphae as in aerial mycelium.

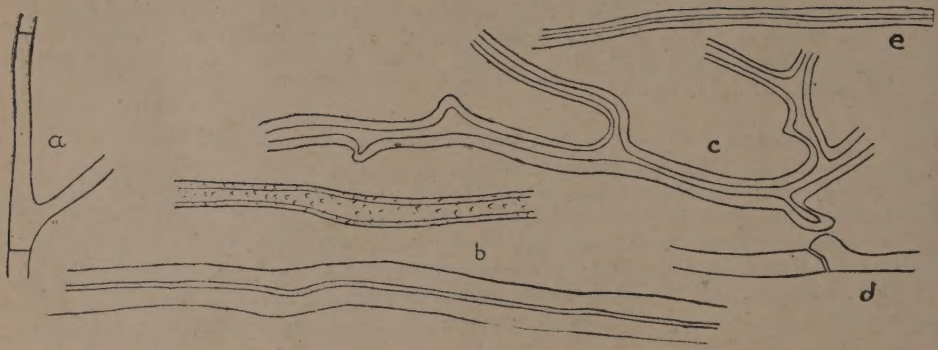
FOMES FOMENTARIUS (L. ex. Fries) Kickx.

(Pl. I, fig. 2 ; Pl. II, fig. 12 and text-fig. 2)

OCCURRENCE. *Fomes fomentarius* is widely distributed in Europe, America, India and Australia. In Europe, the fungus is a strong parasite, attacking beech trees from the age of about 40 years : it enters through wounds and causes a destructive heart-rot (Buchwald, 1930). Post glacial fruit-bodies of the tinder fungus, *Fomes fomentarius*, are known from 16 different localities in Denmark (Buchwald and Hansen, 1934). In India, the fungus is common in the temperate regions of the Himalayas and usually grows on dead standing oaks but also attacks living trees. Besides oaks, it grows on dead wood of walnut (*Juglans regia*), maple (*Acer* spp.) and also on conifers like spruce (*Picea morinda*, and fir (*Abies pindrow*). Once the fungus was collected from a dying ash (*Fraxinus excelsior*).

SPOROPHORE. Fruit-bodies sessile, reflexed, hoof shaped (Pl. II, fig. 12), perennial, small or very large. Upper surface crusty, smooth, thick, hard with concentric zonations, white, brown, grey or greyish black. Context 2 cm. or more, brown, corky, hard ; hyphae thick-walled with narrow lumen, yellow, unbranched (Text-fig. 2,b), 3.5-5.7 μ broad and thick-walled, yellow, branched (Text-fig. 2,c), 1.4-3 μ broad, some show fine projections on walls. Thin-walled, hyaline, branched hyphae (Text-fig. 2,a) 1.4-2.8 μ broad present only in hymenium. Clamp-connections not observed. Hymenial surface grey when fresh, turning ox-blood on bruising and grey or brown on drying. Pores minute, 3-4 per mm. Pore tubes 1.5 cm. or more in one year's layer. stratified, dull brown or white in portions usually plugged. Spores hyaline, elliptic-oblong, 16-18 $\times$ 5 μ (Rea, 1922).

* Growth in all cases refers to radial growth in 7 days.



Text-fig. 2. *Fomes fomentarius*, a, thin-walled hypha in hymenium ; b, thick-walled, unbranched hyphae from context, some with projections ; c, thick-walled, branched hypha from context ; d, thin-walled hypha in culture ; e, thick-walled hyphae in culture. All × 1300.

THE ROT. Decay is usually confined to upper part of the trees, and fruit-bodies are formed all along the main trunk. The wood is turned yellowish white and spongy in which black zone lines are prominent (Pl. II, fig. 12).

FUNGUS IN CULTURE (context culture from sporophore on oak)

(a) Growth characters. Growth fast, about 3-3.4 cm. Mat thin, appressed but at places sub-felty to felty with zonations (Pl. I, fig. 2). Early shades 'cinnamon', 'maize yellow', which deepen with age to 'cinnamon buff, 'light ochraceous buff' and 'ochraceous buff'. Undersurface 'cacao brown', 'liver brown' and 'maize yellow'. Odour in culture oily, almost tallow, may be detected when mycelium is extracted from tube (Badcock, 1939). Growth optimum at 30°C., inhibiting at 38°C. (Cartwright and Findlay, 1942). On gallic acid and tannic acid agars, diffusion zones moderately strong ; growth 2.4 cm. on tannic acid, none on gallic acid. On gentian violet agar, growth good, medium slightly discoloured.

(b) Hyphal characters. *Aerial mycelium* : (a) Hyphae thin-walled, hyaline, branched with few clamp-connections (Text-fig. 2,d), 1.4-2.8 μ broad, (b) hyphae thick-walled, rarely branched, hyaline (in hyaline portions of mat) or brown (in brown areas of mat), 1.4-2.8 μ broad. *Submerged mycelium* : Thin-walled hyphae as in aerial mycelium.

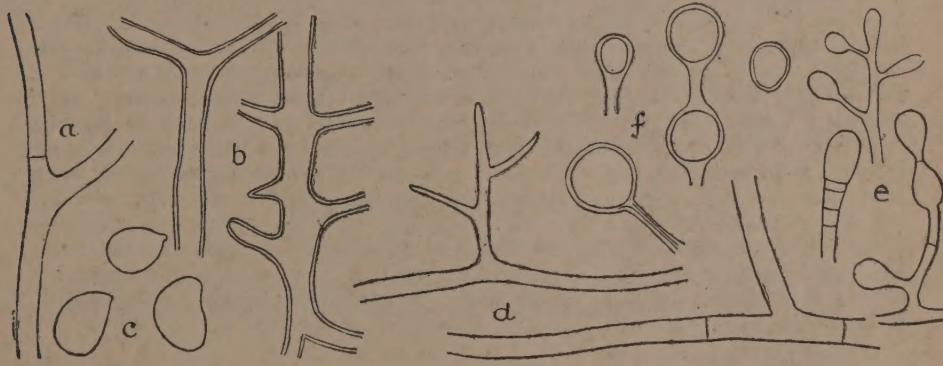
POLYPORUS SULPHUREUS (Bull.) Fr.

(Pl. I, fig. 3 ; Pl. II, figs. 13 and 14 and text-fig. 3)

OCCURRENCE. *Polyporus sulphureus* is widely distributed in Europe and in the tropics and temperate regions in America. In the latter country, the fungus causes a heart rot in many living conifers and hardwoods, particularly oaks and sometimes causes decay in structural timbers (Baxter, 1943). The fungus is able to continue its growth after the tree is felled and the decay of oak in structures can be traced to an infection by the fungus which has already been present in the standing tree (Cartwright and Findlay, 1946). In India, the fungus is common in the Himalayas on living oaks (Pl. II, figs. 13 and 14) as well as on

stumps and sometimes on slash of fir and other conifers. Bose (1946) recorded the fungus from the Eastern Himalayas.

SPOROPHORE. The sporophores appear through wounds on the bark and develop in imbricate clusters (Pl. II, figs. 13 and 14). Fruit-bodies sessile, with a narrow base or sub-stipitate, when fresh, fleshy and exude a pale-yellow juice becoming rigid when dry, usually 5-20×5-25×0.5-2.5 cm. Upper surface sulphur-yellow and bright orange, uneven with a wavy margin. Context light yellow, fleshy when fresh, soft and fragile when dry, 0.4-1.8 cm. thick. Context hyphae thin-walled (Text-fig. 3,a). hyaline with simple septa, much branched, 3.3-12 μ broad, cylindrical thick-walled, profusely branched conducting hyphae (Text-fig. 3,b) which contain the milky juice present in context. Hymenial surface yellow, pores angular, 2-4 per mm. pore-tubes light yellow, straight or slightly oblique, 1-3 mm. long. Basidia 5.5-7 μ in diameter (Lowe, 1934). Basidiospores hyaline, thin-walled, elliptical (Text-fig. 3,c), 6.7-7.8×5 - 5.7 μ.



Text-fig. 3. *Polyporus sulphureus*, a, thin-walled hypha from context ; b, thick-walled conducting hyphae from context ; c, basidiospores ; d, thin-walled hyphae in culture ; e, conidiophores and conidia ; f, chlamydospores. All×1300.

THE ROT. The early stage of the rot is characterised by a yellowish or reddish discolouration and in advanced stages the wood becomes deep red-brown and breaks up into cubical pieces.

FUNGUS IN CULTURE (context culture from sporophore on oak).

(a) Growth characters. Growth 1-1.2 cm., optimum at 30°C., inhibiting at 36°C. (Humphrey and Siggers, 1933). Young mat cottony, becoming woolly later (Pl. I, fig. 3). Pigmentation appears early with shades of 'pinkish buff' and 'capucine buff' which later deepen to 'pale yellow orange' and 'light ochraceous buff'. On gallic and tannic acid agars, diffusion zones absent, growth about 1 cm. on both media. On gentian violet agar, growth sparse, medium not discoloured.

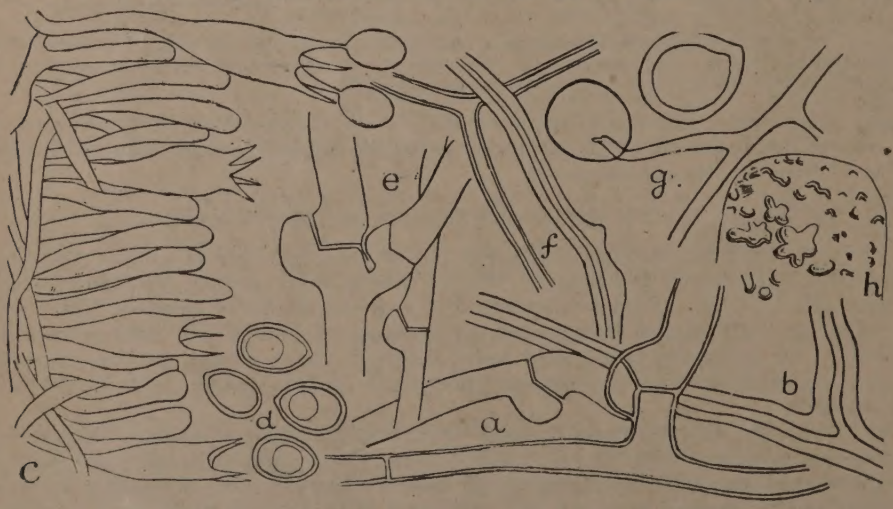
(b) Hyphal characters. *Aerial mycelium.* (a) Hyphae hyaline, thin-walled with simple septa (Text-fig. 3,d), 2.8-9 μ broad ; (b) conidiophores numerous making up most of the aerial mycelium which looks powdery, branched bearing a single conidium at the end of each branch (Text-fig. 3,e) ; (c) conidia numerous, hyaline thin-walled, round or oval, 5-10×5-7.4 μ ; (d) chlamydospores numerous, hyaline slightly thick-walled, terminal or intercalary (Text-fig. 3,f), 11.5-18.5×10-13.2 μ. *Submerged mycelium* : (a) hyphae and (b) chlamydospores as in aerial mycelium.

POLYPORUS OBTUSUS Berk.

(Pl. I, fig. 4 ; Pl. III, figs. 15 and 16 and Text-fig. 4)

OCCURRENCE. *Polyporus obtusus* had so far been known to occur only in North America and hence this is a new record for the fungus in India. In America, the fungus has been recorded principally on oaks but it also occurs on other hardwoods (Riley, 1947). In India *P. obtusus* is parasitic on living oaks, *Quercus semicarpifolia* and *Q. dilatata*, but can also grow saprophytically. It occurs in the Western Himalayas (Chakrata division in the Uttar Pradesh and Bashahr division of the Punjab) between 6,000-12,000 ft. where oaks are common. The soft, thick, sessile, reflexed fruit-bodies are usually formed at some height on the main trunk (Pl. III, fig. 15) or side branches on branch stubs indicating that the fungus is a wound parasite.

SPOROPHORE. Fructifications usually solitary, 5-7 × 5 × 2-3 cm. or as large as 15 × 13 × 5 cm. Sporophores white when fresh, yellow or yellow-brown when dry. Upper surface thickly hairy, soft, velvety. Context white or light yellow, spongy when fresh becoming tough to corky when dry, 2 cm. or more broad. Hyphae thin-walled or slightly thick-walled, hyaline, much branched with abundant clamp-connections (Text-fig, 4,a), 2.1-5 μ broad, and thick-walled with narrow lumen, light yellow in mass but individually hyaline, sparsely branched (Text-fig. 4,b), 3-5.7 μ broad. Hymenial surface white when fresh soon turning yellow. Pores large, irregular (Pl. III. fig. 16), of varying sizes, usually from 0.3-0.6 mm. but may be upto 1 mm. in diameter. Tubes white turning light yellow on drying, equal, straight or slightly oblique. 0.5-3 cm. long. Basidia cylindric (Text-fig. 4.c), 9.8-11.5 × 4.3-5 μ, with 4 sterigmata upto 4.3 μ long, 1.4 μ broad at base' Basidiospores hyaline, oval, slightly thick-walled (Text-fig. 4,d), 5.7-7.5 × 4.3-5.7 μ, average 6.5 × 4.8 μ.



Text-fig. 4. *Polyporus obtusus*, a, thin-walled and slightly thick-walled hyphae from context ; b, thick-walled hypha with narrow lumen from context ; c, hymenial layer in sporophores from nature showing basidia ; d, basidiospores ; e, thin-walled byphae in culture ; f, thick-walled hhphae culture ; g, chlamydospores ; h, fruit-body in culture (diagrammatic) showing platelike structures. All × 1300 except fig. h, × 1μ.

THE ROT. The fungus causes a serious heart rot (white rot). The heartwood is decayed and becomes white and spongy. The resulting white rot continues to advance in the sapwood ultimately causing the death of the tree.

FUNGUS IN CULTURE (context culture from sporophore on oak).

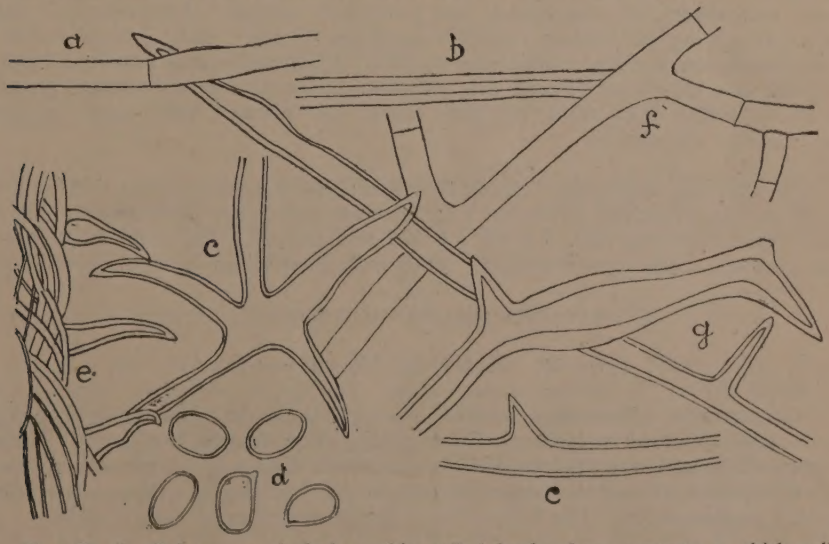
(a) Growth characters. Growth 3.2 cm. Young mat downy in general (P1.I, fig.4), cottony round inoculum becoming woolly to felty with age, white. In tube-cultures, the cottony to woolly mat tends to be felty at upper portion of slant often completely filling tube between agar slant and cotton plug. The mycelium develops a tinge of 'light buff' turning to 'light ochraceous buff' and 'cream buff' in 2 months. Odour in culture moderately fragnant, Jasmine according to Badcock (1939). On gallic and tannic acid agars, diffusion zones moderate, growth 1.5 cm. on tannic acid, none on gallic acid. On gentian violet agar, growth good, medium discoloured.

(b) Hyphal characters. *Aerial mycelium* : (a) Hyphae hyaline, thin-walled, much branched with abundant clamp-connections (Text-fig. 4,e) 2.1-5 μ broad ; (b) hyphae hyaline, thick-walled, branched, aseptate (Text-fig. 4,f), 14-28 μ broad ; (c) chlamydospores hyaline, apical or intercalary, thin-walled (Text-fig. 4,g), 7.8-11.4 $\times$ 5.7-9.3 μ *Submerged mycelium* : (a) Thin-walled hyphae and (b) chlamydospores as in aerial mycelium. *Fruit-body* : Normal, consists of platelike growth from agar (Text-fig. 4,h) instead of regular pore tubes. Basidia and basidiospores as in fruit-body produced in nature.

POLYPORUS CUTICULARIS (Bull.) Fr.

(P1. I, fig. 5 ; P1. III, fig. 17 and text-fig. 5)

OCCURRENCE. *Polyporus cuticularis* is widely distributed in Europe and America and is found fruiting on wounds of maple and other hardwood shade trees and also



Text-fig. 5. *Polyporus cuticularis*, a, thin-walled hypha from context ; p, thick-walled hypha from context ; c, setae hyphae from context ; d, basidiospores ; e, hymenial layer showing setae ; f, thin-walled hypha in culture ; g, setae hyphae in culture. All $\times$ 1300.

on slash. Baxter (1943) remarks that it causes an extensive butt rot in oak and red gum. In India, the fungus has been recorded from the Eastern Himalayas (Bose, 1934) and is also common in the Western Himalayas, in Chakrata division of the Uttar Pradesh, Bashahr and Kulu divisions of the Punjab. It occurs usually on various species of oaks on which the fungus may be parasitic as well as saprophytic. Once it was recorded on a living tree of *Morus alba* in Dehra Dun. The fungus grows on slash of conifers like deodar, spruce and fir.

SPOROPHORE. Fruit-bodies sessile, reflexed, imbricate, soft when fresh, hard and brittle on drying. Upper surface brown to rusty brown, thickly hairy on a thin cuticle, hairs soft in fresh sporophores becoming hard and bristle-like on drying. Context brown, hard, fibrous, fibres running across fruit-body, upto 4 mm. thick. Hyphae thin-walled, yellow or nearly hyaline, sparsely branched (Text-fig. 5,a), 3.5-6.8 μ broad, and thick-walled, yellow, unbranched (Text-fig. 5,b), 4-6.8 μ broad. Setae hyphae present (Text-fig. 5,c), brown. Hymenial surface yellowish brown, pores angular, 3-5 per mm. Pore tubes vertical or slightly oblique, brown, 2-5 mm. long. Basidiospores yellow, oval with an apiculus (Text-fig. 5,d), 5.7-7.1 $\times$ 4.3-5.7 μ, average 6.9 $\times$ 4.8 μ. Setae usually present but may be rare or absent, brownish yellow, thick-walled, base bulbous, pointed and straight or slightly bent (Text-fig. 5,e) 16.5-26.4 $\times$ 6.6-8.3 μ.

THE ROT. The fungus causes a white stingy rot with chocolate coloured to black zone lines in the wood (Pl. III, fig. 17).

FUNGUS IN CULTURE (context culture from sporophore on oak).

(a) Growth characters. Growth moderately rapid, 2.5-3 cm. Young mat cottony becoming thick woolly soon (Pl. I, fig. 5). Pigmentation appears early with shades of 'buff yellow' and 'baryta yellow' which deepen to 'buckthorn brown' with shades of 'clay colour' and 'antimony yellow'. Undersurface 'buff yellow' deepening to 'amber brown', 'capucine orange', 'pale orange yellow'. Growth optimum at 30°C., inhibiting at 38°C. (Cartwright and Findlay, 1942). On gallic and tannic acid agars, the diffusion zones moderately strong, growth 1.6 cm. on tannic acid, trace on gallic acid. On gentian violet agar, growth slow, medium discoloured.

(b) Hyphal characters. *Aerial mycelium* : (a) Hyphae mainly thin-walled, yellow, septate, branched, (Text-fig. 5,f), 2.5-6.7μ broad ; (b) thick-walled, brown, setae hyphae (Text-fig. 5,g), 2.8-5.7 μ broad and setae, 7.1-18.5 $\times$ 2.1-2.8μ sometimes present. *Submerged mycelium* : Thin-walled hyphae as in aerial mycelium.

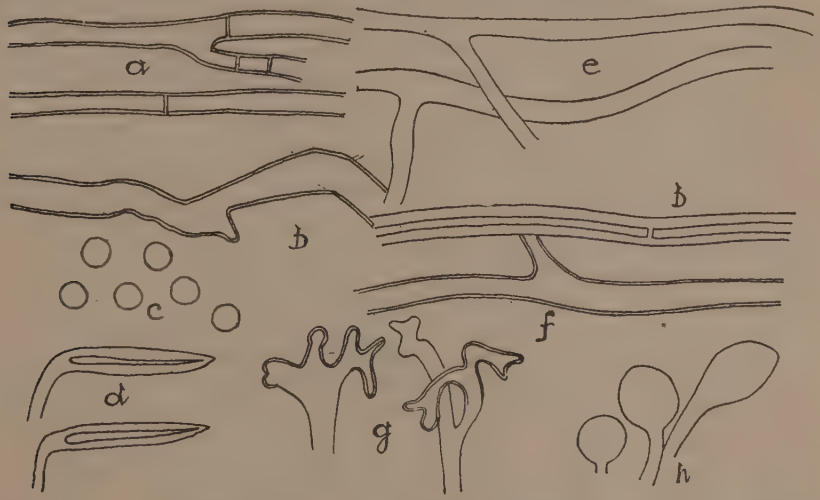
POLYSTICTUS XERANTICUS Berk.

(Pl. I, fig. 6; Pl. III, fig. 18, and text-fig. 6)

OCCURRENCE. *Polystictus xeranticus* occurs in the eastern Himalayas (Lebong-type locality, collected by Hooker), Bose (1946). The fungus is common in the Chakrata division, Uttar Pradesh, Kulu and Bashahr divisions, Punjab, and appears to be distributed all over the temperate zone in the Himalayas. It has not been recorded outside India. The fungus is a common destroyer of slash of oaks.

SPOROPHORE. Fruit-bodies sessile, reflexed, imbricate (Pl. III, fig. 18), thin, light, annual, usually growing closely appressed to the hymenial surface of previous

year's fruit-bodies, but in no organic connection with the latter, coriaceous when fresh, hard and brittle on drying. Upper surface soft, yellow or brown, faintly fzoned. Context thin, 0.3-0.5 mm., golden brown (brownish yellow). Hyphae thin-walled or slightly thick-walled, light yellow, with simple septa, branched (Text-fig. 6,a), 2.1-3.6 μ broad, and thick-walled with narrow lumen, brown, septate, unbranched (Text-fig. 6,b), usually 2.8-4.3 (-7) μ broad. Hymenial surface yellow-brown with a sheen ; pores angular, minute, 4-5 per mm. Pore tubes 1-2.5 mm. long, golden brown. Basidiospores thin-walled, light yellowish, round (Text-fig. 6,c), 2.5-3.5 μ, average 3.0 μ in diameter. Setae numerous, yellowish brown to dark brown, thick-walled, straight, pointed (Text-fig. 6,d), 25-76 $\times$ 5-6.6 μ.



Text-fig. 6. *Polystictus xeranticus*, a, slightly thick-walled hyphae from context ; b, thick-walled hyphae from context ; c, basidiospores ; d, setae ; e, thin-walled hyphae in culture ; f, thick-walled hypha in culture ; g, coralloid hyphae in culture ; h, chlamydospores All $\times$ 1300.

THE ROT. The fungus causes a white pocket rot in the wood surrounded by firm brown areas (Pl. III, fig. 18). The tissues inside pockets become white and very much disintegrated which, in advanced stages of rot, fall away forming cavities separated from each other by narrow partition walls.

FUNGUS IN CULTURE (context culture from sporophore on oak).

(a) Growth characters. Growth slow, about 3-4 mm. Mat felty becoming woolly later (Pl. I, fig. 6). Early shade consists of 'pinard yellow' which deepens to 'light cadmium', 'apricot yellow', 'light orange yellow', 'hay's maroon', 'acajou red'. Undersurface 'carob brown' deepening to 'bister', 'cinnamon-rufous', 'kaiser brown'. On gallic and tannic acid agars, diffusion zones moderate, growth 4 mm. On gentian violet agar, growth moderate, medium discoloured.

(b) Hyphal characters : *Aerial mycelium*. (a) Hyphae thin-walled, hyaline, branched, with simple septa (Text-fig. 6,e), 1-3.2 μ broad ; (b) hyphae slightly thick-walled, light yellow, branched, septate (tex-fig. 6f), 1.7-5.7 μ broad ;

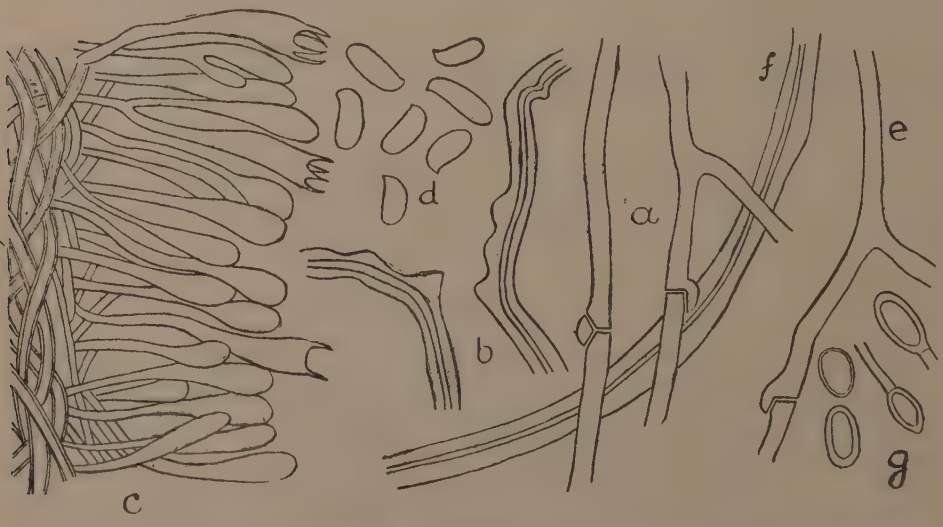
(c) coralloid hyphae thick-walled, yellow-brown (Text fig. 6,g) ; (d) chlamydospores hyaline, thin-walled, terminal (Text fig. 6,h), 10-21.4 × 10.7-14 μ. *Submerged mycelium* : As in aerial mycelium, but thick-walled hyphae and chlamydospores rare.

TRAMETES DICKINSII Berk.

(Pl. I, fig. 7 ; Pl. III, figs. 19-21 and text-fig. 7)

OCCURRENCE. *Trametes dickinsii* appears to be restricted to the far east. The type locality is Japan. In India, it was first reported by Bose (1937) from Lokra hills (Assam) and Bihar. The fungus is extremely common on stumps of oaks (Pl. III, fig. 19) in the Chakrata division in Uttar Pradesh.

SPOROPHORE. Fruit-bodies sessile, imbricate, usually reflexed (Pl. III, fig. 19), applanate (Pl. III, fig. 20), coriaceous. Upper surface cream coloured when young becoming brownish black with age, concentric zonations prominent, ill defined or absent. Context thin, 3-4 mm., 'capucine buff' to 'light ochraceous salmon'. Hyphae mostly thick-walled with lumen large or small, unbranched (Text-fig 7,b), pale coloured (pale yellow), 2.6-3.2 μ. Thin-walled, hyaline branched hyphae with few clamp-connections (Text-fig. 7,a), 1.4-2.6 μ broad, present only in the hymenium. Hymenial surface biscuit coloured, pores round to angular or tending to be labyrinthiform, 2-3 per mm. Pore tubes long, upto 2 cm., penetrating to different depths in the context, old tubes filled with a white lint like material which comes out in form of thread. Basidia clavate (Text-fig. 7,c), 7.1-9.5 × 4 μ with sterigmata upto 4 μ long. Basidiospores hyaline, thin-walled, oblong with an apiculus (Text-fig. 7,d), 5.7-7.1 × 2.1-2.8 μ, average 6.1 × 2.7 μ.



Text-fig. 7. *Trametes dickinsii*, a, thin-walled hyphae from hymenium ; b, thick-walled hyphae from context ; c, hymenial layer showing basidia ; d, basidiospores ; e, thin-walled hypha in culture ; f, thick-walled hypha in culture ; g, chlamydospores. All × 1300.

THE ROT. The fungus is a common slash decaying organism. White elongated pockets filled with disintegrated fibres and separated by firm, brown areas appear in the rotted wood (Pl. III, fig. 21). White to light cream coloured mycelial sheets appear in the crevices in the wood.

FUNGUS IN CULTURE (Spore culture of the fungus from oak).

(a) Growth characters. Growth moderately rapid, 2-2.5 cm. Mat felty becoming woolly later with raised piles of aerial hyphae in zonations (Pl. I, fig. 7), white. On gallic and tannic acid agars, diffusion zone absent, growth 1.2 cm. On gentian violet agar, growth good, medium not discoloured.

(b) Hyphal characters. *Aerial mycelium* : (a) Hyphae thin-walled, hyaline, branched with clamp-connections (Text-fig. 7,e), 0.7-2.8 μ broad ; (b) hyphae thick-walled with narrow lumen, unbranched (Text-fig. 7,f), 1.4-4.3 μ broad ; (c) chlamydospores thin-walled, hyaline, mostly terminal (Text-fig. 7,g), 10-16.5 $\times$ 8.2-10.7 μ. *Submerged mycelium* : Same as aerial mycelium. *Fruit-body* : Appears readily with pores well defined, circular, elongated or labyrinthiform, but probably abortive.

SUMMARY

Seven fungi causing important diseases and decays of oaks in India have been described as to their occurrence in India and elsewhere, characters of the sporophores, and the type of rot they produce in the wood. Biology of these fungi have been studied in culture.

Stereum princeps Jungh., *Fomes fomentarius* (L. ex Fries) Kickx., *Polyporus sulphureus* (Bull.) Fr., *Polyporus obtusus* Berk. and *Polyporus cuticularis* (Bull.) Fr. have been found to attack living species of *Quercus*, and also cause decay of felled logs, stumps and converted timber. *S. princeps*, *P. sulphureus* and *P. obtusus* are usually wound parasites and fruit-bodies of these fungi develop on cankers, fire scars, broken branch stubs, etc. *P. obtusus* has been described for the first time in India.

Polystictus xeranticus Berk. and *Trametes dickinsii* Berk. are important as destroyers of slash.

We wish to thank Professor S.R. Bose of Calcutta and Miss E. M. Wakefield of the Royal Botanic Gardens, Kew, for help in identification of fungus sporophores which were frequently sent to them.

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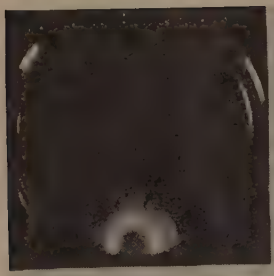
Explanation of Plates

Plate I .. Figs. 1-7. 14 days old culture on malt agar at 24°C. in dark. Fig. 1. *Stereum princeps*, Fig. 2. *Fomes fomentarius*, Fig. 3. *Polyporus sulphureus*, Fig. 4. *Polyporus obtusus*, Fig. 5. *Polyporus cuticularis*, Fig. 6. *Polystictus xeranticus*, Fig. 7. *Trametes dickinsii*, Fig. 8. Sporophores of *Stereum princeps*, on a decayings tump of *Quercus* sp., Fig. 9. Sporophores of *S. princeps* on a fallen log of oak showing hymenial surface.

Plate II .. Fig. 10 Sporophores of *S. princeps* on a fire scar of a living *Quercus semicarpifolia*, Fig. 11. Rot in oak due to *S. princeps* (× $\frac{1}{3}$), Fig. 12. Sporophore of *Fomes fomentarius* (left) and the rot on oak (right) produced by the fungus. Note the black zone lines in the wood (× $\frac{1}{3}$) Fig. 13. Sporophores of *Polyporus sulphureus* on wounds and scars of a living *Quercus semicarpifolia*, Fig. 14. Sporophores of *P. sulphureus* on a stump of *Abies pindrow*.

Plate III .. Fig. 15. Sporophore of *Polyporus obtusus* on scar of a living *Quercus semicarpifolia*, Fig. 16. Sporophore of *P. obtusus* showing hymenial surface (× $\frac{2}{3}$),

Fig. 17. Rot in oak produced by *Polyporus cuticularis*. Note the zone-lines in the wood (× $\frac{1}{3}$), Fig. 18. Sporophore of *Polystictus xeranticus* (left) and the rot on oak (right) produced by the fungus (× $\frac{1}{3}$), Fig. 19. Sporophores of *Trametes dickinsii* on an oak stump, Fig. 20. Young sporophores of *T. dickinsii* (× $\frac{1}{3}$), Fig. 21. Rot on oak produced by *T. dickinsii* (× $\frac{1}{3}$).



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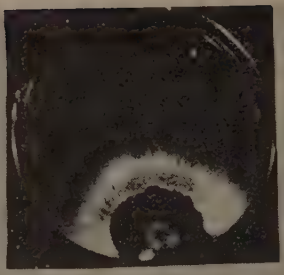

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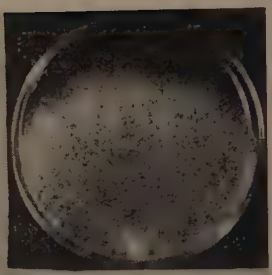

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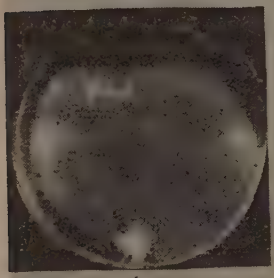

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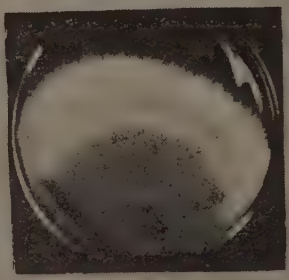

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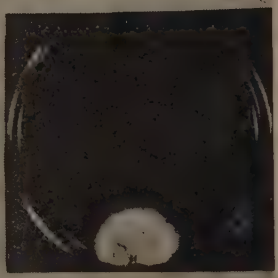

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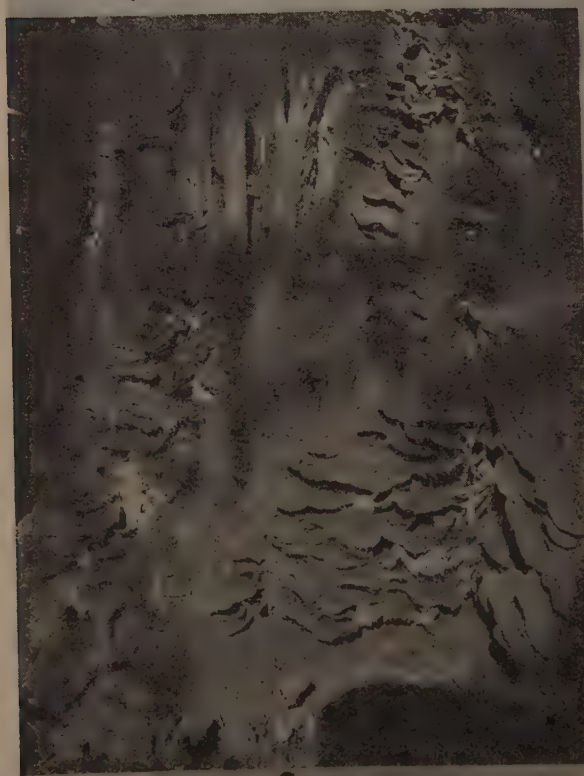

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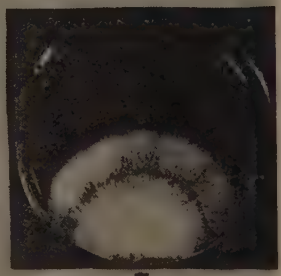

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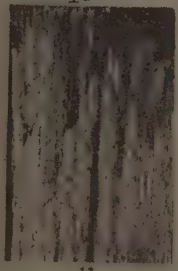

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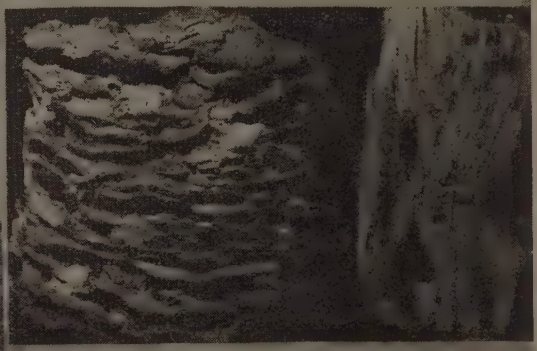

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A NEW SPECIES OF GLOEOSPORIUM ON ANTHOCEPHALUS CADAMBA

Akshaibar Lal and I. N. Tandon

(Accepted for publication November 15, 1950)

Introduction

ANTHOCEPHALUS CADAMBA is a large deciduous tree growing wild in Northern and Eastern Bengal, and the Western Coast; and cultivated in Northern India as a garden tree for its ornamental flowers.

The tree is attacked by a fungus which forms spots on the leaves, the younger twigs and the flowers.

The disease is most conspicuous during the months of January and February. Leaves fall off, twigs dry up, and the entire branch or its affected part assumes an appearance of dry, dead, leafless twigs resembling the "Die back" and "Twig blight". In severe cases even complete defoliation may occur.

The attack begins immediately after the new leaves come up. The first indication of the disease is the appearance of yellowish minute specks which soon turn into reddish-brown spots. The spots are of the size of a pin head which gradually increase in size and attain a diameter of .1 to 1 cm. The spots gradually turn brown and finally to a dark-brown or tan colour, and are more or less irregular in shape. The infected tissues dry up and collapse causing a depression at the centre of the spot. The spot is found to be surrounded by a distinct dark coloured margin. Very often two or more adjacent spots run together and form irregular lesions, involving the whole leaf. On the upper surface, and sometimes on the lower, there may often be seen a series of narrow concentric rings of alternate light or deeper shade especially near the margin. The fructifications are present on both the surfaces, arranged in concentric rings or scattered over the whole spot. Their number varies greatly in accordance with the prevailing humidity and temperature. They are black in colour, the older ones are salmon coloured due to the discharge of spore masses.

When the leaf spots are numerous, there is a pronounced chlorosis of the intervening leaf tissue. Callus is formed at the junction of the leaf with the petiole, resulting in defoliation of the tree.

Lesions are also formed on the petioles, twigs and fruits. The affected fruit first shows a greenish yellow area which gradually turns into a dead, brownish, sunken spot. (Plate I, 3). Gradually the whole fruit is affected which shrinks and mummifies (Plate I, 4) and, finally, may fall off or remain hanging on the twig.

Materials and Methods

The material used for various studies was isolated from the leaves, stems and fruits and was found to be identical in every case. The organism was purified by single spore culture.

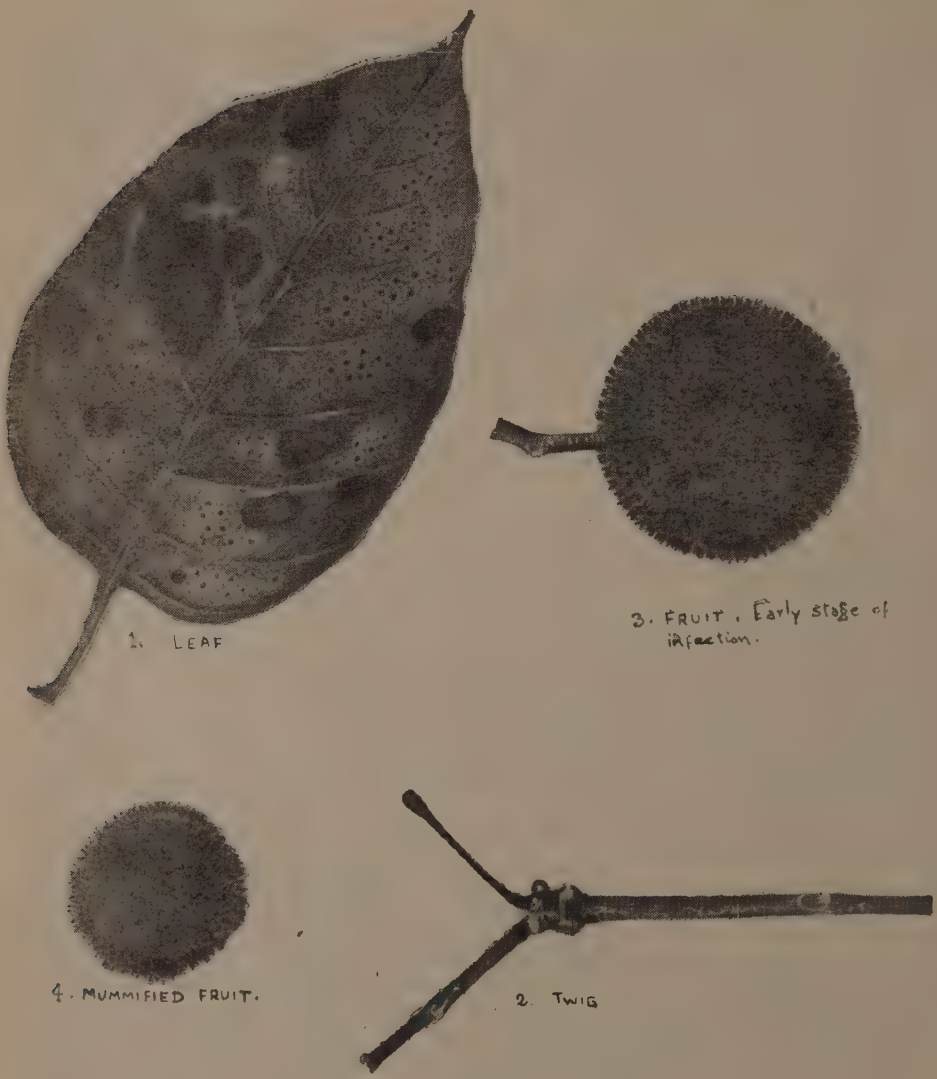

PLATE. 1. SYMPTOMS OF THE DISEASE.

Plate 1 : Symptoms on (1) Leaf (2) Twig (3) Fruit early stage of infection and (4) Mummified fruit.

For microtome sections the material was fixed in Craf and the blocks were prepared in the usual way. The sections were stained in aqueous solution of magdella red and light green in clove oil. Hand sections were stained with cotton-blue in lacto-phenol and mounted in the latter and sealed by means of zinc cement. Stem pieces were softened by boiling in 5% KOH before cutting section.

MORPHOLOGY OF THE PARASITE IN THE HOST

The mycelium of the parasite is localised in the tissues of the discoloured parts, entering directly into the cells and rapidly killing them causing the appearance of sunken spots. Within the host it is composed of irregularly septate, thick-walled, brown hyphae. Younger hyphae are thin-walled, hyaline, and sparingly septate while the older ones are brown, thick walled, tortuous with septa at short intervals. They are often irregularly swollen or even nodular, and contain granu-

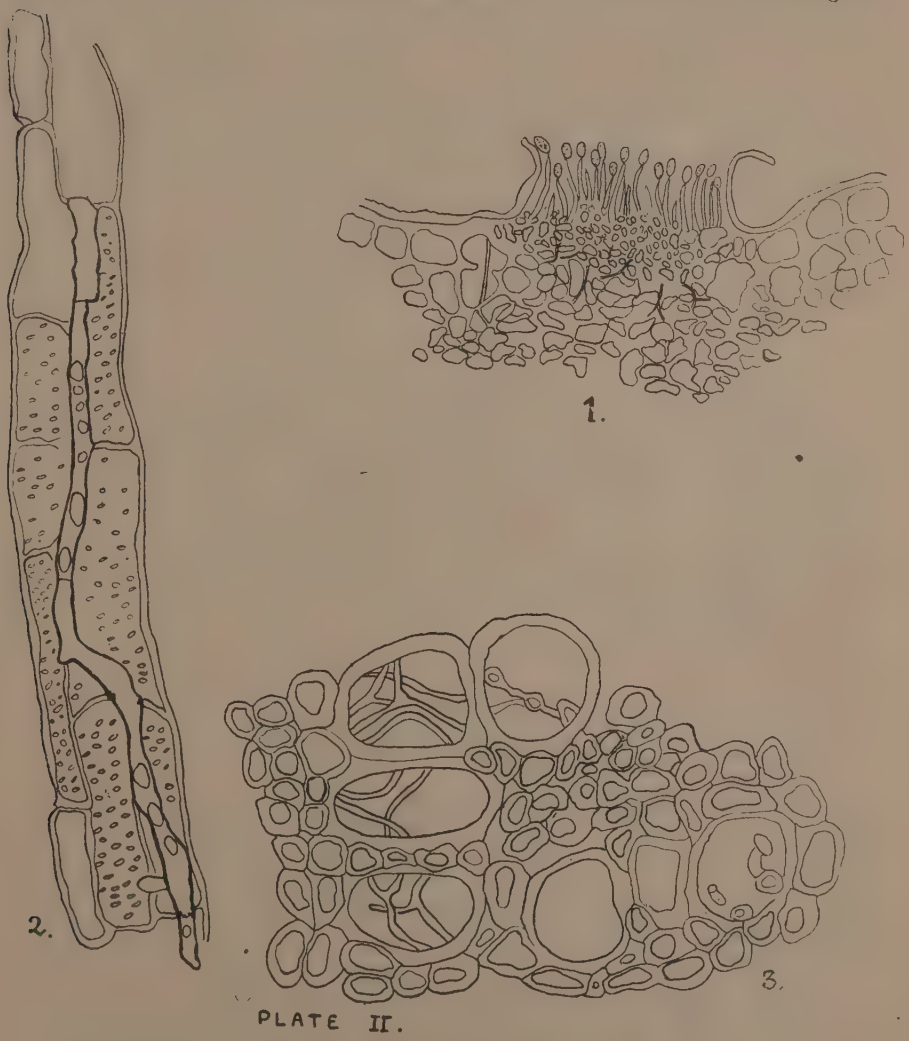

PLATE II.

Plate II .. 1. An acervulus from a T.S. of leaf.
2. L.S. of stem showing old septate mycelium.
3. T.S. Stem showing old septate mycelium.

lar protoplasm and numerous oil globules. The latter type is common in the older portions of twigs, while the hyaline ones are usually found in leaves. Mycelium is intracellular. In the stem, fibro-vascular bundles are invaded. In the vessels (Plate II, 2), very thick hyphae occur which measure 1.5 to 6.0 μ. Thick walled, dark coloured chalamydospores are sometimes formed inside the vessels (Plate II, 3). They are more or less spherical and measure 6.0 to 9.0 μ.

Mycelium accumulates below the epidermis raising up the latter into small pustules, by the growth of stromatic layer of fungal cells. From the surface of this layer, short, cylindrical, hyaline, unbranched and unseptate conidiophores arise, which measure from 10 to 31.5 μ in length. The conidiophores bear conidia at their apices. The epidermis is ruptured and the surface of the spot becomes covered with salmon coloured masses of spore. Presence of setae has not been observed. The diameter of the acervuli ranges from 74 to 185 μ (Plate II,1.) Conidia are hyaline individually, but in mass they look salmon coloured. When young they are full of protoplasmic granules and contain generally two oil globules. They are unicellular, often divided by a median septum before germination, varying from cylindrical to oblong in shape with rounded ends. The spores vary greatly even in the same acervulus. The measurements of 1000 spores at random gave a variation of 6 to 10 μ in length and 3 to 4 μ in width, with an average of 8.5×3.5 μ.

PATHOGENICITY

Inoculation on the host: Inoculation experiments were performed in young seedlings grown in pots. Two different methods of inoculation were followed. After surface sterilization, plants were either sprayed with spore suspension in sterile water, or culture of the fungus containing both spores and mycelium was placed on the plant parts by means of a sterilized needle, and wet cotton was placed over it. The plants were kept inside a moist glass chamber.

In another series, plants were inoculated by both the methods described above, but they were kept outside and not inside the glass chambers. Some of the plants were slightly scratched by means of a sterilized needle. Upper as well as lower surfaces of the leaves were inoculated. The results are summarised below :—

(1) Infection occurs almost equally on both the surfaces. The presence or absence of stomata, therefore, makes little difference.

(2) Under moist condition, the percentage of infection is higher than in the outside dry atmosphere, and the infection occurs earlier under moist condition than under dry condition. This fact has been further confirmed by observations in different seasons. In the hot season, when the atmosphere is dry, infections are rare, but are common after a shower of rain. This explains the severity of infection during monsoon months.

(3) The kind of inoculum made no difference, as infection occurred readily with mycelium as well as spore suspension.

(4) It was found that the infection occurred readily in unbruised parts of the plant, thus indicating that the organism can penetrate directly into the host.

Artificially inoculated spots were found to be exactly similar to those occurring in nature.

The comparision of the measurements of the spores of this fungus indicated that species of *Gloeosporium* recorded on *Lycopersicum esculentum*, *Solanum melongena* and *Capsicum annuum* lies near about that of the present species, and, hence, inoculations were performed on these hosts but none of these took infection. *Gloeosporium fructigenum* (Berk.) obtained from I.A.R.I., New Delhi, also did not infect *Anthocephalus* seedlings. This fungus, therefore, has been regarded as a new species and named as *Gloeosporium anthocephali*. The characters of the species are as follows :—

Spots irregular, often circular with a depressed centre, reddish brown, margin dark, 0.1 to 1 cm. in diameter, but may be larger after coalescence ; Acervuli amphigenous, dark, follicolous or caulicolous, sub-epidermal, ranging in diameter from 74 to 184 μ ; conidiophores short, cylindrical, hyaline, unseptate unbranched, 10 to 32 μ in length ; conidia minute, cylindrical to oblong with rounded ends, unicellular, hyaline, but salmon in masses, 6-10 $\mu \times 3$-4 μ.

Habitat : In leaves, stems, and fruits of *Anthocephalus cadamba* Roxb ; Banaras (November 1945), Allahabad, Dehradun. Type in College of Agriculture, Banaras Hindu University, Banaras.

Maculis irregularibus, saepe circularibus, 0.1-1 cm. latis, rubro-brunneis late brunnes marginatis, depressis ; Acervulis amphigenis, obscurioribus, foli-Caulicole, sub-epidermicis, dien superficialibus, epidermide dehiscentecinctis, 74-185 μ diam ; conidiophoris brevissimis, Cylindraceus, hyalinis, Continuis, aramosis, 10-32 μ longis, Conidiis, minutis treti-oblongis, untrinque rotundatis, continuis, hyalinis, salmo in massa, 6.0-10.0 $\mu \times 3$ to 4 μ.

Hab :—in foliis, caulibus, et fructibus *Anthocephalus cadamba* Roxb. Banaras Allahabad, Dehradun. Typus in College of Agriculture, Banaras Hindu University, Banaras.

MODES OF PERENNATION OF THE DISEASE

The fungus over-summers either in leaves and twigs remaining on the surface of soil, or in diseased twigs attached to the trees. Viability of the fungus in each case has been tested up to 8 to 10 months by means of spore germination tests, as well as by successful inoculations of young *Anthocephalus* plants. Presence of the chlamydospores inside the twigs suggests that the fungus may also over-summer in this form.

DISSEMINATION OF THE FUNGUS

Dissemination of anthracnoses has usually been attributed to rain and moisture. The spores are washed down from the diseased twigs and trickle on the leaves, which are infected. This is further confirmed by the following observations :—(a) The diseased spots on the leaves generally appear by the beginning of the rainy season, that is, by the end of June. (b) Due to abnormal rains in the second week of April, 1945, some diseased spots were observed just two days after the rains. (c) A single branch consisting of 20 leaves was marked and it was found that they were infected after rain.

Thus, it is obvious that rain and moisture are necessary for the dissemination of the disease.

GERMINATION OF SPORES

Germination in water : The conidia readily germinate in tap water, within 8 to 12 hours. The germ tube grows out near one end, rarely from both ends, and occasionally from the middle of the spores ; often the spores become one septate just before germination.

Germination in culture : Brownish, thick walled, globose to irregular chlamydospores are freely formed not only on the germ tube but on the mycelium as well. They may be intercalary or terminal, in chains or single. Conidia of the normal type also develop being borne singly on short stalks.

The time required for germination of spores varies with the culture medium used, temperature and hydrogen-ion concentration. (1) Spores which germinate slowly in distilled water do so more quickly when placed in 2-5% sugar solutions. (2) Hanging-drop culture studies showed that the germ tubes attain maximum length at 30°C. Below and above this, there is a decrease, both in the percentage of germination and in the average length of germ tube. (3) Highest percentage of spore germination is obtained at pH 7.5. Above and below this, the percentage of germination decreases. The average length of germ tube follows closely the percentage of germination.

Germination on leaf (*Plate III*) : The upper surface of a leaf was sprayed with suspension in sterile water. Within 24 hours after spraying, a great majority of the spores produced germ tubes either from one end or both the ends. The germ tube is short, but when it comes in contact with the epidermis of the host, it swells up at its end into a thick walled, angular or roundish, brown enlargement, the appressorium, which becomes thin and colourless on one side, and from this spot a germ tube appears. This infection hypha penetrates through the cuticle into the epidermal cell, probably by mechanical pressure as reported by Dey[1] for *Colletotrichum lindemuthianum*. Stomatal entrance or penetration through wounds has not been noticed. The appressorium in *Gloeosporium* has been described as chlamydospore by Biraghi[2], because of its ability to form a new hypha even when it does not come in contact with the host.

FACTORS AFFECTING SPORULATION

(1) Continuous light or darkness has been found to inhibit sporulation, while alternate light and darkness encourages sporulation. (2) Conditions of high humidity favour sporulation whereas low humidity retards it. (3) Sporulation was found to be more at a temperature lower or higher than the optimum (30°C). (4) Sporulation is poor in rich media while it is good in poor

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[1] Dey, P.K. ... Studies in physiology of parasitism. Infection by *Colletotrichum lindemuthianum*. *Ann. Bot.* **33** : 305-312.

[2] Biraghi, A (1934) ... Sul signifacto biologico dei presunte appressori nel gen. *Gleosporium*. *Boll. R. Staz. pat. Veg.* N.S. xiv, **2** : 202-210. (R.A.M. 13 ; 782, 1934)

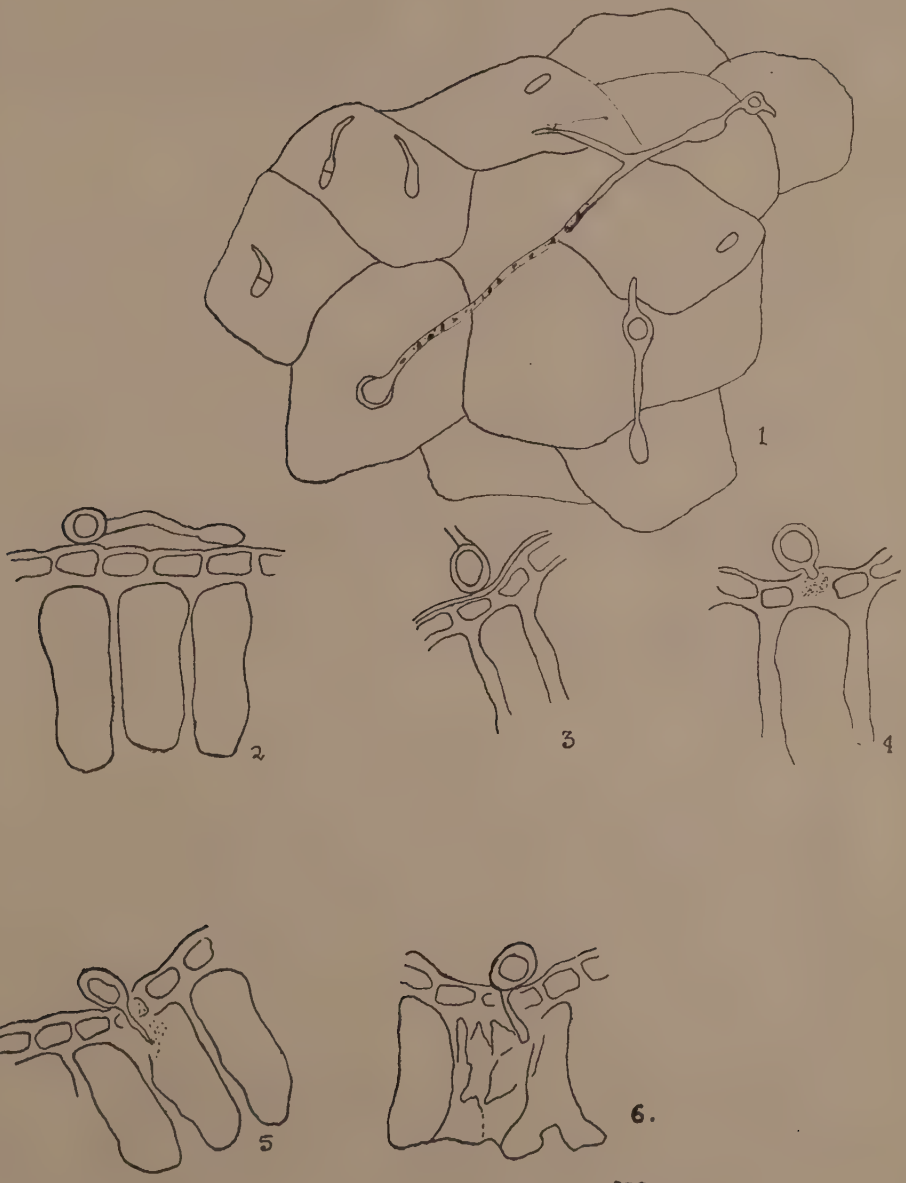

PLATE III.

Plate III .. 1. Different stages of germination of spores on leaf tissue.
2-6. Formation of chlamydospores and the penetration of infection hypha inside the leaf tissue.

media. (5) Sporulation is accelerated in culture along the lines of wounding. (6) The size of conidia has been found to vary with different media. The average length and breadth is greatest in potato dextrose and turnip juice agar, and least in Brown's starch agar. It is further observed that the spores formed in culture are greater in size as compared to those formed on the host fissue. Obviously the richness of the medium is responsible for their size.

SUMMARY

Gloeosporium sp. is parasitic on leaves, petioles, stems and fruits of *Anthocephalus cadamba*. Symptoms of the disease and the morphology of the parasite have been described.

The fungus has been shown to be a new species of *Gloeosporium* and is named as *G. anthocephali.*

Penetration of the infection hypha in the leaf tissue is always accompanied by the formation of an appressorium.

The fungus perennates in dead twigs attached to the trees, as well as in the detached twigs and fruits which remain lying on the surface of the soil. Dissemination is favoured by rain and moist weather.

Light, humidity, temperature, media and wound have been found to influence sporulation in culture.

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THE QUANTITATIVE DETERMINATION OF MOULDS IN SWEETPOTATO AND WHEAT FLOURS

M. L. GATTANI

(Accepted for publication November 20, 1950)

METHODS designed principally to assay the number of bacteria in flour have been described by Kent-Jones and Amos (1940) and Barton-Wright (1938). Christensen (1946) has described methods for the determination of mold content of wheat flour and concluded that the composition of the medium on which the flour is cultured, the technique of making the dilutions, and the method of counting the number of colonies in the culture dishes were three variables which influenced the number of molds cultured from a given flour sample. He found malt-salt medium to be superior to the other five media tested for determining the number of molds in flour, and showed that a "higher mold count is obtained from a given sample of flour, using malt-salt medium, if the flour is suspended in sterile saline solution and this suspended in agar, than if it is similarly suspended and cultured on the surface of the agar or if it is spread dry on the surface of the agar."

Sweetpotato flour mixed with wheat flour has in recent years been supplied to the consumers in India. The present studies were undertaken at the instance of the Ministry of Food to determine the mold content of sweetpotato and wheat flour samples.

EXPERIMENTAL

Five culture media were used for the assay of these samples. They were potato dextrose agar, acid potato dextrose agar, pH 5.5, Christensen's malt-salt agar*[1], Smith Humfeld agar*[2] and potato dextrose agar containing 3 mols. of sodium chloride per litre. The procedure adopted for assaying was essentially the same as recommended by Christensen. Five grams of flour sample were weighed out on a piece of sterile paper. This was placed in a three oz. screw-capped medicine bottle and 0.5% sodium chloride solution was added to make upto 50 cc. Usually, in each case 47·5 cc. of 0.5% sodium chloride were required

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*[1] *Christensens malt salt medium.*

Malt extract	20.0 gm.
Sodium chloride	75.0 gm.
Agar	20.0 gm.
Distilled water to mak ...	1.0 litre.

*[2] *Smith Humfeld agar.*

$NaNO_3$	1.0 gm.	KH_2PO_4	1.0 gm.
Dextrose ...	10.0 gm.	Agar	15.0 gm.
Distilled water to make ... 1.0 litre.			

for this purpose. Fifteen sterile marble beads were then added to the mixture and the whole shaken vigorously. Before any of the flour had settled, and immediately after the vigorous shaking, 5 cc. of the turbulent liquid were transferred to a second 3 oz. bottle to which subsequently 45 cc. of sterile 0.5% NaCl solution were added. The bottle was shaken and 1 cc. aliquots were transferred to sterile petri plates. In this way, replicate plates of 1 :100 dilutions were made.

The five culture media were cooled to 45°C after autoclaving for twenty minutes at 15 lbs. pressure. 20 cc. of each medium were poured out in the sterile petri-plates containing 1 cc. aliquots of 1 : 100 dilutions of the flour sample. Twenty replicate plates were used for each sample and they were incubated at 25°C for three days. Counting of the colonies was done according to the method suggested by Christensen (1946). Samples of sterile flour were assayed from time to time as a check on the number of mold colonies that may enter from the air or other sources. For this purpose the flour sample was sterilised by heating it slowly to 180°C in a gas oven, stirring it occasionally to prevent caking, after which it was left at 180°C for several hours. The sterile flour was assayed in the same way as the other flour samples. Replicate dilutions of 1 :100 sterile flour seldom averaged more than one colony per ten plates and, therefore, it was concluded that the number of mold colonies entering from the air was negligible.

Eight samples of flour were assayed on the five media. Both potato dextrose agar and Smith Humfeld agar supported bacterial growth, and assay plates containing these media gave only few colonies of *Rhizopus* or *Aspergillus* sp. On PDA+NaCl medium, only *Aspergillus* sp. colonies were obtained. On acidified PDA, both *Rhizopus* and *Aspergillus* colonies were obtained, and on malt-salt medium, in sample no. 3, few colonies of *Fusarium*, and in the case of sample no. 4 few colonies of *Fusarium* and *Alternaria* sp. were also obtained. It is, therefore, apparent that the flour samples were rich in *Rhizopus* and *Aspergillus* sp. flora. Common species of *Aspergillus* were *A. niger*, *A. glaucus*, and *A. candidus*. It is noteworthy that *Penicillium* spp. were absent in the flour samples.

Results of the counts of mould colonies obtained from the various samples on acidified PDA, PDA+Nacl and malt-salt agar are summarized in Table I.

TABLE I

Counts of mold colonies per gram of the flour sample on different media

Sample No.	Composition of the sample	Acidified PDA				PDA+NaCl				Malt salt medium			
		Rhizopus	Aspergillus	Other Fungi	Total	Rhizopus	Aspergillus	Other Fungi	Total	Rhizopus	Aspergillus	Other Fungi	Total
1	100% sweet potato	420	220	...	640	...	1,400	...	1,400	140	620	...	760
2	100% sweet potato	640	340	...	980	...	1,360	...	1,360	300	675	...	975
3	100% Atta (Wheat flour)	360	380	...	740	...	1,620	...	1,620	180	740	10	930
4	95% Atta (Wheat flour) 5% sweet potato	260	420	...	680	...	1,660	...	1,660	120	900	30	1,050
5	100% sweet potato	560	400	...	960	...	1,440	...	1,440	250	700	...	950
6	100% Atta (Wheat flour)	160	680	...	840	...	1,580	...	1,580	80	970	...	1,050
7	95% Wheat flour & 5% Sweet potato	460	650	...	1,110	...	1,640	...	1,640	350	810	...	1,160
8	100% Sweet potato	540	590	...	1,130	...	1,700	...	1,700	240	930	...	1,170

It would appear from the table that, in case of every sample, acidified PDA gave the highest counts of *Rhizopus* colonies. No *Rhizopus* colonies developed from any sample on PDA+NaCl, and the number of *Rhizopus* colonies developing on malt-salt agar was lower than that developing on acidified PDA. With regard to the counts of *Aspergillus*, it will be seen that each sample gives increased counts of *Aspergillus* sp. by 250% to 400% when it is cultured on PDA+NaCl than when it is cultured on either acidified PDA or malt-salt agar. On malt-salt medium the counts of *Aspergillus* colonies were higher than those on acidified PDA. The colonies of *Fusarium* and *Alternaria* obtained in case of sample no. 3 and 4 on malt-salt medium are negligible in comparison to the number of *Aspergillus* or *Rhizopus* colonies.

In order, therefore, to get an idea of the total number of mold colonies per gram of each of the flour samples, it would seem reasonable to add the total number of *Rhizopus* colonies developing on acidified PDA to the total number of *Aspergillus* colonies developing on PDA+NaCl. If one were to make counts only on malt-salt medium the number of mold colonies developing from 1 gm. of flour sample would be very low. For example, sample 1 gives a total mold count of 760 mold colonies per gram of the flour on malt-salt medium, whereas the actual number of molds developing from one gm. of this sample is 420 of *Rhizopus* and 1400 of *Aspergillus i.e.*, 1820 colonies.

The percentage increase in mold counts when the number of molds is determined by counting the total number of *Rhizopus* colonies on acidified PDA and the total number of *Aspergillus* colonies on PDA+NaCl over the number of colonies counted on malt-salt medium are given in Table II.

TABLE II

Mold counts per gram of the flour sample on malt-salt medium and the actual number of moulds in the sample.*

Sample No.	Mold counts on malt salt medium	Actual No.	Percentage increase
1	760	1,820	240
2	975	2,000	205
3	930	1,980	210
4	1,050	1,920	183
5	950	2,000	210
6	1,050	1,740	163
7	1,160	2,100	181
8	1,170	2,240	191

It will be seen that the actual number of molds, as determined by the counts of *Rhizopus* and *Aspergillus*, separately on acid PDA and PDA+NaCl, is far greater than the number of molds as counted on malt-salt medium. The increase varies from 163% to 240% in case of the different samples.

DISCUSSION

It has been shown by Christensen (1946) that the number of mold colonies, developing from any given wheat flour sample, is influenced by the method of

*Actual number of molds was determined by adding the number of *Rhizopus* colonies from acid PDA medium and *Aspergillus* colonies from PDA + NaCl medium.

making the dilution, the medium on which the flour is cultured, and the method of counting the colonies in the petri dishes. He found malt-salt medium superior to other media tested in his studies.

In the present studies with samples of sweet potato, wheat, and mixture of sweet potato and wheat flours, it has been found that the eight flour samples abounded in *Rhizopus* and *Aspergillus*. Highest counts of *Rhizopus* colonies were obtained on acid potato dextrose agar, whereas potato dextrose agar containing 3 mols. of sodium chloride gave highest counts of *Aspergillus*. On this medium, *Rhizopus* was suppressed completely. Thus, a fairly accurate count of the mold colonies developing from 1 gm. of flour sample is given by adding the number of *Rhizopus* colonies from acidified PDA and the number of *Aspergillus* colonies from PDA containing 3 mols. of sodium chloride. If the number of mold colonies is determined by taking into consideration the counts from only one medium *i. e.*, malt salt agar, the number represented would be very low, which is actually not the case. The increase in percentage of the number of mold colonies, obtained by counting the total number of *Rhizopus* colonies from acidified PDA and the *Aspergillus* colonies from PDA containing 3 mols. of NaCl, over the number of colonies counted on malt-salt medium, as has been stated, may greatly vary. It is, therefore, concluded that the total number of mold colonies from a flour sample can better be estimated by culturing the sample on selective media giving the maximum number of colonies of predominant fungi, rather than by counting the number of colonies on one particular medium.

The author is grateful to the Head of the Division of Mycology, for his criticism and helpful suggestions. Thanks are also due to Mr. D. V. Karmarkar, Technical Adviser, Ministry of Food, Govt. of India, for supplying flour samples.

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BACILLUS SUBTILIS CAUSING SOFT ROT OF MANGO

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(Accepted for publication November 20, 1950)

THE mango fruit, when quite ripe, is subject to rot caused by several fungi and bacteria. Patel and Padhye (1949) have shown that *Bacterium carotovorum* is one of several such organisms responsible for it. Although, in literature, spore forming, rot producing organisms have been occasionally reported, it is almost an accepted fact that these spore formers are really not plant pathogens in true sense, since almost any organism can grow on ripe banana, tomato, and mango fruits owing to their high sugar content. Elliott (1930) has recorded about 15 spore forming organisms attacking different plants, but, these being insufficiently described, Bergey (1949) *et al* consider them to be of a doubtful status under the genus *Bacillus*. The genus *Bacillus*, as conceived by Bergey *et al*, is meso—or thermophilic, and comprises mostly of saprophytes and, as such, is not retained in phytopathogenic bacteriology.

The works of Jones (1901), Dowson (1941), Waldee (1945), Patel and Padhye (1949) and others show conclusively that the true plant pathogens causing rot in several plants are not spore formers. Those which produce rotting in plants are contaminants since they rarely, if at all, attack healthy plant tissues having little or no sugars, as in carrot, cucumber, peas, beet, radish, brinjal, etc.

The writers while working with rot producing organisms of fruits isolated a mesophilic, spore forming, gram-positive organism whose description is given below :—

Morphology. The bacterium is a rod with rounded ends. In 96-hour old culture grown in nutrient broth at 30°C, the size of the cells is 2.18 (2.6-1.35) × 0.69 (0.81-0.54)μ. They are motile with peritrichous flagella, creamy white, gram-positive, non-capsulated, not acid fast, spore formers, no involution forms, single or in chains, and stain readily with common dyes such as methylene blue and carbol fuchsin.

Cultural characters. On nutrient agar slants, a fair, smooth, dull, filiform opalescent, yeast like growth with pale olive buff colour occurs in 96 hours, with no distinctive odour. On potato dextrose agar slants, the growth is good, smooth, glistening, dull in the spreading area, while the central portion is opalescent, filiform with irregular margins, pale olive gray colour and slight yeast-like odour in 96 hours. In nutrient broth, the growth is cloudy with pellicle, no sediment or floccules even after 7 days. Litmus is completely reduced after 10 days with no floccules or sediment, but the pellicle is present. In plain milk, the pathogen grows abundantly clearing the milk with no sediment and no tyrosin crystals. The pathogen is an strict aerobe. The optimum temperature for growth lies about 25°-30°C, the maximum 45°C and minimum 5°C. The thermal death point is 95°C.

Biochemical reactions :—Liquefaction of gelatin begins on the second day and proceeds rapidly. Tests made with strips of filter paper impregnated with lead acetate showed no production of hydrogen sulphide after 7 days. The Gore's method shows no reaction for indol production. Nitrite is formed after 5 days, while ammonia is not produced. In Cohn's medium, the growth is slight, becoming cloudy after 7 days. In Uschinsky's medium, growth is slight, whitish, with a pellicle, and no fluorescence in 7 days. The growth is positive in Koser's uric acid medium. Acetyl methyl carbinol is not produced.

Carbohydrates :—The organism gave fair to moderate growth in dextrose, xylose, lactose, sucrose, raffinose, mannitol, and salicin with no gas and no sediment, but distinct acid in dextrose, xylose, sucrose, and slight or no acid in raffinose, mannitol and glycerine, with thick pellicle in all. Starch is hydrolysed. On Sismmon's citrate medium, the growth is good, dull yellowish. On potato cylinder, the growth is copious, dull brown, not flowing, and darkening the entire surface in 5 days. Casein is digested. The pathogen grows on media with pH ranging from 5.3 to 9, but the best growth occurs at 6.28 pH.

Pathogenicity :—In repeated trials, it was found that the organism was pathogenic on mango, banana, green and ripe tomato, while infection was not produced on cucumber, carrot, *bhendi*, French beans, cowpea, radish, brinjal, onion, garlic, sweet-potato, potato, peas, beet, elephant foot, cauliflower, apple, lemon, *mosambi* and chillies.

It will be seen from the description given above that the organism under reference resembles *B. subtilis* in several respects. It may be of interest to note here that Madhok and Din (1943) isolated an organism from tomato, which they named *B. fructodestruens*, although it belonged to *B. subtilis* group. Had they inoculated other fruits, or used other organisms for comparison and for inoculation of tomato fruits, possibly the necessity of adding one more spore forming organism to the ones already in confusion would have been avoided.

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STUDIES ON SOME INDIAN SYNCHYTRIA
I. FOUR NEW SPECIES FROM BIHAR

R. C. Lacy

(Accepted for publication December 31, 1950)

THE genus *Synchytrium* includes species parasitic on a large number of hosts, some of which are of great economic importance. Mhatre and Mundkur (1945) gave an account of the Indian species of *Synchytrium*, based on an examination of herbarium material deposited in the *Herb. Crypt. Ind. Orient.* at New Delhi. Recent studies by Cook (1945) on this interesting group of chytrids have, however, shown that well-preserved or relatively fresh material showing the structure of the galls is of great importance in ascertaining the identity of the species. Identity of the species based merely on the size of the hypnospores in old herbarium material may prove to be erroneous.

Collections of *Synchytrium* species were made in Patna, Bihar, and the neighbouring places, in the month of September, when the moist warm weather conditions favoured abundant development of the fungus. The species which have been collected are being studied cytologically. In the present paper, an account of the morphology of the fungi, pathological symptoms produced on the host, and identity of the species are presented.

1. Synchytrium species on Stereospermum suaveolens DC

Stereospermum suaveolens DC. is an ornamental garden plant, the leaves of which were found to be infected with a *Synchytrium*. The presence of the fungus infection appears as an orange-yellow patch in the form of tiny little protuberance, up to 0·5 mm. in diameter. Sections through the infection patches revealed the presence of prosorus and resting spores produced in the galls. The fungus developed within the epidermis, completely filling the enlarged cell. In due course, the surrounding cells proliferated, so that the prosori or the resting spores were surrounded by stout pointed hairs. The gall protruded above the epidermal surface and opened by a fissure, indicating the place where the enveloping cells were united above the resting spores.

The gall of the prosorus ranges from 360-540 μ in diameter. The prosorus is 100-200 μ in diameter and the sporangia measure 20-22 $\times$ 24-28 μ. The resting spores are reddish-brown in colour with thick granular content. They are thick-walled, from 8-11 μ in thickness. The wall of the host is non-persistent and flakes away. The mature resting spores measure from 84-118 μ in diameter and are situated in galls measuring from 112-224 μ in diameter.

Synchytrium stereospermi Lacy sp. nov. Galls chiefly hypophyllous, simple, or compound by coalescence, formed above the epidermis and surrounded by sheath of conical upturned hairs. Prosorus 100-200 μ in diameter, sporangia rectangular or polygonal, thin-walled. Resting spores filling up the host cell, reddish-brown, thick-walled, 84-118 μ in diameter, wall up to 11 μ.

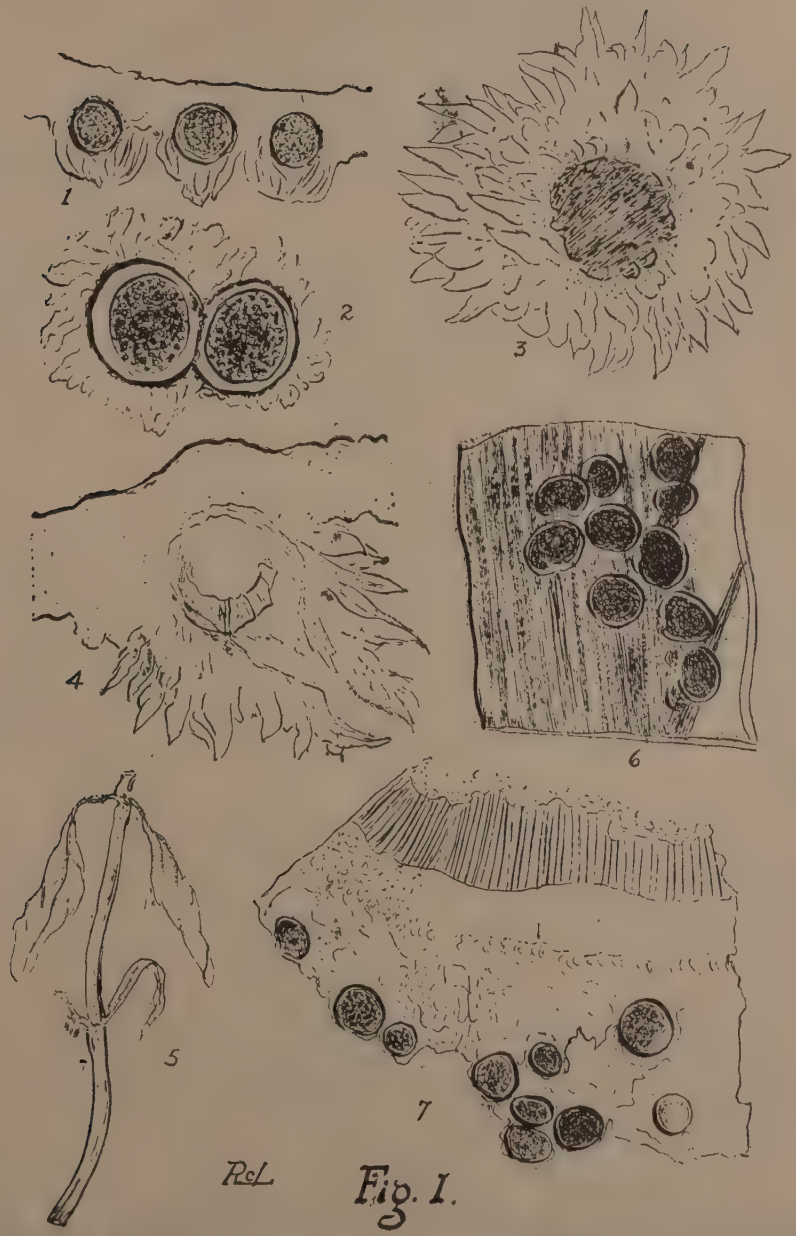

Figure I. Synchytrium stereospermi: 1. Cross-section of leaf of *Stereospermum suaveolens* showing resting spores and galls, × 75.
2. Surface view of galls and resting spores, × 150.
3. Surface view of gall of prosorus, × 150.

4. Section of the gall and empty prosorus, × 150.

Synchytrium sesamicola : 5. An infected shoot of *Sesamum indicum.* Natural size.

6. Surface view of resting spores on a piece of host tissue, × 75.

7. Cross-section of stem, showing resting spores in the galls, × 75.

Hab. on leaves of *Stereospermum suaveolens* DC., Patna, Bihar (India), 12-9-1950, leg. R. C. Lacy (Type). Type deposited in *Herb. Crypt. Ind. Orient.* New Delhi, India.

Gallis communiter hypophyllis, simplicibus, vel in coalescentia compositis, super epidermidem evolventibus, vagina circumdatis, conicalicalium capillorum erectorum. Pressori 100-200 μ in diam. Sporangiis rectangularibus vel polygonalibus, tenuis parietibus. Sporis quiescentibus rubro-fuscis, parietibus crassis, 84-118 μ in diam., hospitem cellulam implementibus. Parietibus usque ad 11 μ.

Hab. in foliis *Stereospermum suaveolens*, DC.

2. SYNCHYTRIUM SPECIES ON SESAMUM INDICUM

Collections of self-sown *Sesamum* plants in the fields revealed the occurrence of a *Synchytrium* species parasitic on young shoots. Infection was confined to young axillary shoots which became malformed considerably. Instead of showing normal, robust growth, they appeared like curled, spindly growth with deformity. Due to imperfect leaf expansion, the young leaflets were puckered and curled into curious shapes. Severely infected shoots did not bear any flowers or capsules, but withered away prematurely. Since there was no *Sesamum* crop nearby, the present observations are restricted to a study of the disease on self-sown plants. Therefore, it has not been possible to ascertain the economic importance of the disease.

Infection was chiefly confined to young stems, petiole and veins of the leaves presenting a warty brown or rugose appearance of the infected parts. Most of the infected shoots that were found on the plant parts were at the base and at close proximity to the ground. Infection on mature stems was sparse and scattered while, on young shoots, it was continuous due to coalescence of the pustules.

Microscopic studies revealed only the presence of the resting spores and no prosorus or sporangial stage has been observed. The young resting spore is developed within the hypertrophied epidermal cell and coalescence of galls in juxtaposition appears to form compound galls. Hypnospores range from 78-126 μ and are situated in galls ranging from 154-168 μ.

Synchytrium sesamicola Lacy, sp. nov. Galls mostly on the stems, petiole and veins ; grayish-brown, presenting a rugose appearance, usually simple, or compound when in close juxtaposition. Galls formed above epidermal surface, surrounded by proliferating, hypoplastic cells. Prosorus not observed. Resting spores filling up the host cell, spherical, 78-128μ in diameter, wall upto 6μ in thickness.

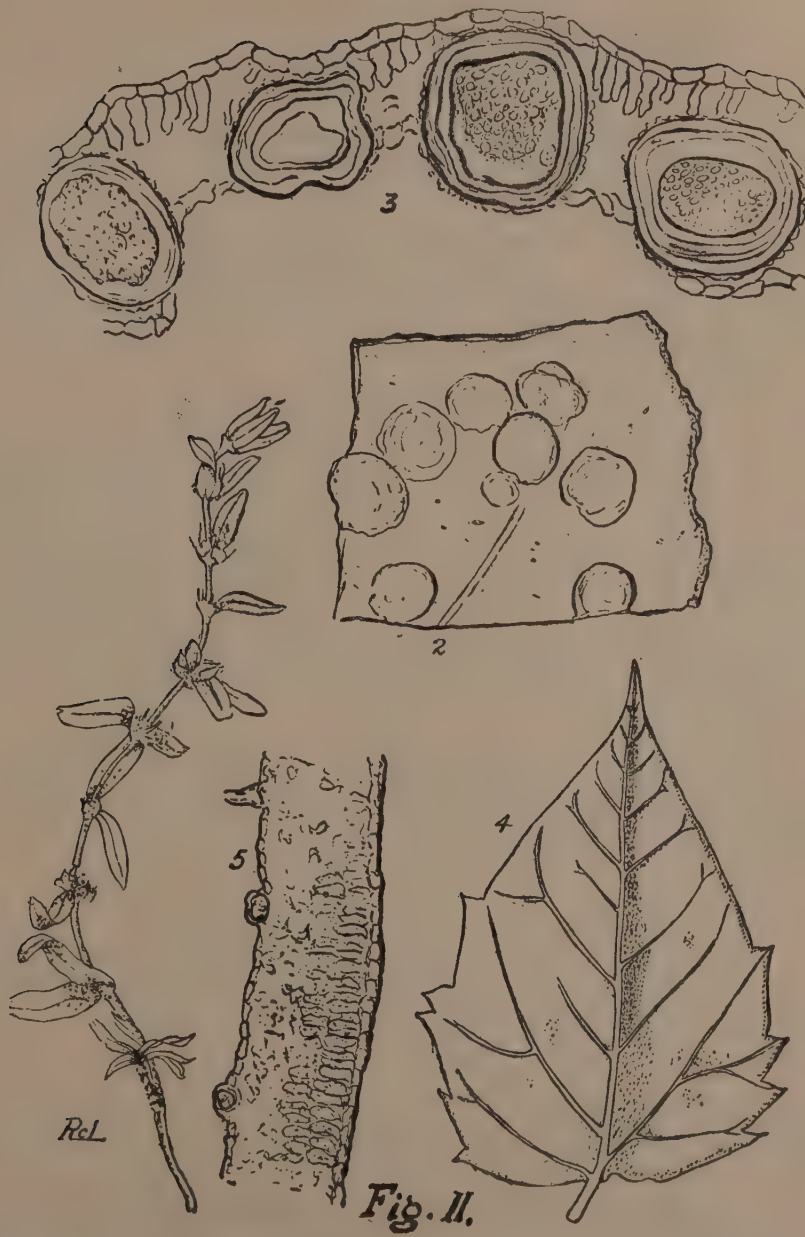

Figure II. Synchytrium borreriae : 1. Young shoot of *Borreria hispida* showing infection Natural size.

2. Surface view of resting spores on leaf, × 75.

3. Cross-section of leaf showing relationship of galls, resting spores and host tissue, × 150.
Synchytrium nyctanthidis: 4, Ventral view of leaf of *Nyctanthes arbor-tristis* showing a few patches of infected erea. Natural size.
5. Cross-section of leaf showing simple galls and resting. spores, × 150.

Hab. on the stems and petioles of *Sesamum indicum* L., Patna, Bihar (India), 4-9-1950, leg. R. C. Lacy (Type). Type deposited in *Herb. Crypt. Ind. Orient.*, New Delhi, India.

Gallis praesertim in caulibus, petiolis et venis ; cinereofuscis, rugosis, communiter simplicibus vel compositis, si in densa juxtapositione. Gallis supra-epidermatibus formatis, hyperplastis cellulis proliferantibus circumdatis. Pressori non visa. Sporis quiescentibus sphericis hospitem cellulam implentibus, 78-128 μ in diam., parietibus usque ad 6 μ crassis.

Hab. in caulibus petiolisque *Sesamum indicum* L.

3. SYNCHYTRIUM SPECIES ON BORRERIA HISPIDA

Borreria hispida K. Sch., a member of the Rubiaceae, is a wild weed growing in dry waste lands. After the monsoons, several of the plants showed orange-yellow specks, on the upper surface of the leaves, like tiny pin points when examined with a hand lens. Microscopic examination revealed the occurrence of a *Synchytrium* species in the resting-spore stage. The young resting spores formed within the epidermis. As the infected cell enlarged, it was pushed inwards and, therefore, the resting spore appeared in sub-epidermal position. There was very little or no protrusion of the gall above the epidermal layer. The galls range from 154-210 μ in diameter. There was no thickening of the leaf tissue in the infected region.

The resting spores were large, separate and showed thick-walled condition. The outermost layer formed by the host tissue was thick, yellowish-brown and persistent. Mature spores measure 112-154 μ in diameter and possess granular oily contents. Germination of the resting spore has not been observed. The thickness of the wall ranges from 11-28 μ and is generally three-layered.

Synchytrium borreriae Lacy, sp. nov. Galls on the leaves, chiefly hypophyllous, appearing as orange-yellow specks, primarily epidermal in origin, later innate and sub-epidermal, not causing proliferation or leaf-thickening. Sporangia not observed. Resting spores sub-globose to spherical, reddish-brown, 112-154 μ in diameter, thick-walled, wall upto 28 μ thick. Wall of the host firm and persistent.

Hab. on the leaves of *Borreria hispida* K. Sch., Patna, Bihar (India), 12-9-1950, leg. R. C. Lacy (Type). Type deposited in *Herb. Crypt. Ind. Orient.*, New Delhi, India.

Gallis in foliis, praesertim hypophyllis, sicut citreo-flavis maculis (minutis), generaliter epidermalibus in ortu, postea epidermalibus proliferationem non causantibus, neccrassa folia. Sporangia non visa. Quiescentibus sporis sub-globosis vel sphericis, rubro-fiscis, 112-154 μ in diam., parietibus usque ad 28 μ crassis. Parietibus, hospites cellulae firmis et persistentibus.

Hab. in foliis *Borreria hispida* K. Sch.

4. SYNCHYTRIUM SPECIES ON NYCTANTHES ARBOUR-TRISTIS

Nyctanthes arbor-tristis L. is a shrubby tree grown in gardens for its fragrant flowers. During the humid weather of September, a *Synchytrium* species was found parasitising the leaves. The infection spots were very minute and inconspicuous, so that the presence of the fungus in fresh material became apparent only by the orange pigment of the resting spores. In older material, it was found very difficult to locate the site of the infection spots, since the leaves also showed infection by an *Oidium* species.

Microscopic examination revealed the presence of the resting spores developing within the epidermal cells. The infected cell enlarged several times the normal host cell, and the resting spore completely filled the host cell. There was no proliferation or division of the neighbouring cells, so that the resting spore was lodged in an enlarged epidermal cell (about 112 μ in diam.), showing the simplest type of gall formation. When the adjacent cells showed infection, each one of them was distinctly separate without any compound gall formation. The resting spores were 48-72 μ in diameter, with relatively thin wall, approximately 5 μ thick.

Synchytrium nyctanthidis Lacy sp. nov. Galls on living leaves, appearing orange-yellow, minute; resting spores in hypertrophied epidermis, completely filling it, thick-walled, pale cinnamon-brown, 48-72 μ in diameter, wall upto 5 μ thick.

Hab. on leaves of *Nyctanthes arbor-tristis* L., Patna, Bihar (India), 1-9-1950, leg. R.C. Lacy (Type). Type deposited in *Herb. Crypt. Ind. Orient.*, New Delhi, India.

Gallis in foliis vivis. citreo-flavis, minutis. Sporis quiescentibus in hypertropha epidermide, perfecte implentibus, crassis parietibus, pallide cinnamon-fuscis, 48-72 μ in diam., parietibus usque ad 5 μ crassis.

Hab. in foliis *Nyctanthes arbor-tristis* L.

In conclusion, the writer wishes to acknowledge his thanks to Dr. M.J. Thirumalachar for his help, and to Rev. J. Bressers, S J., Ranchi, for correcting the Latin diagnosis of the new species.

SUMMARY

In the foregoing paper are described four species of *Synchytrium* parasitic on higher plants; namely, *Synchytrium stereospermi* on *Stereospermum suaveolens*, *Synchytrium sesamicola* on *Sesamum indicum*, *Synchytrium borreriae* on *Borreria hispida* and *Synchytrium nyctanthidis* on *Nyctanthes arbor-tristis*. These fungi were collected in the vicinity of Patna, Bihar, in the month of September, after the rains, and are proposed here as new species.

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TWO NEW PHYSIOLOGIC RACES OF SPHACELOTHECA SORGHI

SYED VAHEEDUDDIN

(Accepted for publication December 20, 1950)

POTTER (1915) demonstrated the distinct difference between covered and loose kernel smuts of sorghum by their behaviour on several varieties of sorghum. Kulkarni (1918) inoculated seeds of different strains of milo sorghum with chlamydospores of *Sphacelotheca sorghi* and *S. cruenta*, and concluded that dwarf milo is susceptible to loose smut and resistant to covered smut. Potter and Melchers (1918) inoculated different commercial varieties of *Sorghum vulgare* with spores of *S. sorghi* and pointed out that some of the varieties were highly resistant. Reed and Melchers (1925) inoculated 250 varieties of sorghum with spores of *S. sorghi* and concluded that Shallu, Sorgos, Broom corn and Kafirs were very susceptible, Sudan grass moderately susceptible, and milo and feterita very resistant.

Tisdale, Melchers and Clemmer (1927) pointed out that milo, hegari and feterita were attacked by kernel smut. They believed the smut on milo and hegari to be a distinct physiologic race, but they were not sure whether it was a race of *S. sorghi* or a hybrid between *S. sorghi* and *S. cruenta*. They also pointed out that the kernel smut on feterita was a distinct race of *S. sorghi* Melchers, Ficke and Johnston (1930) designated these races as races 1, 2 and 3. They pointed out that race 1 does not attack milo, hegari and feterita ; race 2 attacks milo and hegari but not feterita ; race 3 attacks feterita and feterita hybrids, and milo. They (1932) later added two more physiologic races of *S. sorghi*, thus making a total of 5 physiologic races.

The writer (1938, 1942) obtained a new physiologic race (race 6) of *S. sorghi*. at St. Paul, Minnesota (U.S.A.), by inoculating seeds of different host varieties of sorghum with spores of a collection, which was the F_2 progeny of a cross between two biotypes of a single smut sorus. This physiologic race (race 6) differed from those 5 races described previously by Melchers, Ficke and Johnston.

Kernel smut collections of *S. sorghi* from various taluqas (counties) of Hyderabad State (India), were mixed with sorghum seeds of differential host varieties imported from United States of America*, and sown at Government Main Agricultural Experimental Station, Himayatsagar, Hyderabad, in triplicate randomised rod rows in the years 1941-42 and 1942-43.

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* The writer wishes to acknowledge his gratitude to Dr. E. C. Stakman, Chief of the Section of Plant Pathology, University of Minnesota, and Dr. L. E. Melchers, Professor of Plant Pathology, Kansas State College of Agriculture, for the supply of sorghum seed of differential host varieties.

When the earheads emerged, the smutted heads, after taking observations, were picked and burnt to avoid spreading of the spores on the Farm and to prevent introduction of new physiologic forms. Three collections of smut (one from Raichur, one from Gulbarga and one from Aurangabad) were used for two years to see whether they behave differently in different years, and it was noticed that their reaction was almost the same on the differential host varieties for both the years, and they were classified in the same form in both the years.

When the data regarding percentage of infection on the differential host varieties was analysed statistically, it was found that some of the smut collections could be classified under the physiologic forms 1, 3 and 6, while good many collections were different in behaviour from the known 6 forms and, hence, they are classified into two new forms (form 7 and form 8). The old key which was prepared by the writer for identifying 6 physiologic forms is now modified in order to identify these two new forms (form 7 and 8) along with the 6 old ones.

The Key is as follows:—

Key for identifying physiologic forms of grain smut of sorghum [*Sphacelocheca sorghi* (Link) Clinton]

A. Kafir × Feterita (H.C. 2423) resistant
B. Dwarf yellow milo (K.B. 2515, Dwarf yellow milo (C.I. 332), Bierce Kaferita (K.B. 2547), Feterita × Kafir (F.C.I. 8917) and Feterita (S.P.I. 51989) resistant

C. Reed Kafir (C.I. 628) resistant
D. White yolo (K.B. 2525) resistant
E. Shrock resistant .. .. Form 8

E.E. Shrock highly susceptible .. .. .. Form 7

D.D. White yolo (K.B. 2525) susceptible .. .. Form 4

C.C. Reed Kafir (C.I. 628) susceptible
D. Shrock and Hegari (K.B. 2513) resistant .. Form 1

D.D. Shrock and Hegari (K.B. 2518) susceptible
E. Dwarf yellow milo (K.B. 2515), Dwarf yellow milo (C.I. 332) and White yolo (K.B. 2525) resistant .. .. .. Form 6

E.E. Dwarf yellow milo (K.B. 2515), Dwarf yellow milo (C.I. 332) and White yolo (K.B 2525) susceptible .. .. Form 2

A.A. Kafir × Feterita (H.C. 2423) susceptible
B. Pierce Kaferita (K.B. 2547) and Feterita × Kafir (F.C.I. 8917) resistant .. Form 5

B.B. Pierce Kaferita (K.B. 2547) and Feterita × Kafir (F.C.I. 8917) susceptible .. Form 3

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PESTALOTIA PSIDII PAT. ON GUAVA

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(Accepted for publication September 1, 1950)

THE guava (*Psidium guajava* L.), originally a native of South America, is an important fruit crop in India. In the State of Bombay, it occupies an area of 4200 acres of which half is in the districts of Poona, Dharwar, Nasik, and East Khandesh. The plantations are generally located near big cities where there is an assured market.

The trees are usually free from diseases except a fruit canker, caused by *Pestalotia psidii* recorded for Bombay by Chibber (1912), and reported from Mysore by Narasimhan (1939) ; a bark canker due to *Physalospora psidii* Stevens and Peirce, noted by Uppal, Patel and Kamat (1934) in Dharwar and Ahmedabad Districts. *Pestalotia psidii*, although known for a very long time, has not been investigated so far. In August, 1948, guava fruits showing rough, raised, circinate pustules with crater-like depressions in the centre, were received from Chembur (Thana District), Dharwar, and Poona and an ivestigation was, therefore, undertaken.

Symptoms

The disease generally occurs on green fruits and rarely on leaves. Cankers on the green fruits of different varieties exhibit considerable differences in their appearance. For instance, those on "Lucknow 49" are larger, more elevated and numerous ; on "Dholka", not as well developed ; insignificant and inconspicuous on "Sind", whereas "Nasik" variety seems to be almost immune. Ripe fruits are rarely attacked.

The first evidence of infection on fruits is the appearance of minute, brown or rust coloured, unbroken, circular, necrotic areas which, in advanced stages of infection, tear open the epidermis in a circinate manner. The margin of lesions is elevated and a depressed area is noticeable inside. This crater-like appearance is more noticeable on fruits (Plate 1, Fig. 1) than on leaves. The canker is confined to a very shallow area and does not penetrate deep into the flesh of the fruit. The marketable value of heavily infected fruits is greatly impaired as such fruits fetch little or no value in the market.

In older cankers, tufts of white mycelium consisting of numerous spores are noticeable.

Isolation of the Fungus

Guavas with young cankers were washed in water. After air drying they were either dipped in 95% alcohol, or alcohol was applied to infected areas to remove oily material ; they were then flamed lightly to remove any excess alcohol and dipped in a 1 in 1000 solution of corrosive sublimate for 2-3

minutes, and washed in three changes of water. The fruit was then pierced with a pair of flamed forceps and, by means of a sterilised scalpel, the portion of the canker below the rind removed and pieces aseptically transferred to plates containing potato dextrose agar. The plates were inverted and incubated at 26-27°C for 3-4 days. The typical colonies of the fungus were transferred to tubes containing potato dextrose agar.

PATHOGENICITY OF THE FUNGUS

Preliminary trials with detached fruits, both ripe and unripe, failed to give positive results in the repeated inoculation experiments. This might have been possibly due to the fact that the ripe fruits are generally non-susceptible, while green fruits began to ripen quicker when detached. The method was, therefore, suitably modified and inoculations made on fruits still on trees at various stages of their development. This procedure proved quite successful and was followed in all the subsequent experiments.

Fresh, young, green guava fruits on trees were disinfected by 1 in 1000 solution of corrosive sublimate and washed with 2-3 changes of distilled sterilised water. A 2-week old culture was used for inoculation of fruits which were slightly wounded at several places by a sterile needle. Bits of mycelium were placed on wounds which were covered by pieces of damp absorbent cotton. Finally, the whole fruit was covered by cellophane bags to prevent drying of the wounds and the inoculum. Some fruits were inoculated without wounding. Controls were provided in all cases.

The infection was noticeable after 4 days when minute, round, dark brown specks appeared on wounded inoculated green fruits. These specks developed into young pustules 2 days later. The development of these pustules continued for 15 days when tearing of epidermis was noticed. In the case of the green fruits with unwounded surface and the wounded ripe fruits, small specks were noticeable after 8 days, and pustules after 10 days from the time of inoculation. However, no tearing of epidermis was noted in the latter case.

Symptoms on artificially infected fruits were similar in all respects to those resulting from natural infection. Controls remained healthy. The fungus was easily re-isolated from the infected fruits and its pathogenicity re-established.

Only two leaves showing infection were gathered in the orchard, since artificial infection on the leaves was invariably unsuccessful. It may, however, be noted here that sporulation on these two leaves was visible only when dry, indicating that the green leaves although infected may not produce the symptoms and thus act as carriers. (Plate 1, fig. 2)

The results of infection at different stages of fruit are recorded in table I.

TABLE I

Comparative severity of infection by P. psidii *at different stages of fruit development*

Stage of fruit	Severity of infection		Incubation period	
	Wounded	Unwounded	Wounded	Un-wounded
Green fruit	Severe	Moderate	4 days	8 days
Ripe fruit	Moderate	Nil	8 days	Nil
Control	Nil	Nil	Nil	Nil

It will be seen from table I that the fungus is primarily a wound parasite, since the wounded surface of green and ripe fruits showed higher degree of infection than that on unwounded fruits. This is in conformity with the finding of several authors quoted by Guba (1932) who state that spp. of *Pestalotia*, as a group, are weak parasites and, as such, require some injury for infection. In nature, this may be brought about by fruit flies, guava mealy scale, and the *Arbela* caterpillar.

VARIETAL RESISTANCE

To test the varietal resistance of guava, several inoculation experiments were done at different times using green small, green large and ripe fruits of "Lucknow 49", "Kothrud", "Dholka", "Sind" and "Nasik" varieties. Inoculations were made as described before. Controls were provided in each case. The results as recorded in table II were taken 15 days after inoculation.

TABLE II

Varietal resistance of guava fruit to P. psidii

Variety	Severity of infection
1. Lucknow 49	Fruits completely infected, well developed pustules.
2. Kothrud	,,
3. Dholka	Pustules of comparatively small size.
4. Sind	Small pustules.
5. Nasik	Nil

It will be seen that "Lucknow 49" and "Kothrud" varieties are highly susceptible while "Nasik" was highly resistant to infection (Plate I, fig. 3). The controls remained healthy.

MORPHOLOGY OF THE FUNGUS

The fungus produces profuse mycelial growth on the culture media such as Richards agar, potato dextrose agar, lima bean agar, etc. The mycelium of young cultures is subaerial, serrated, thin, septate, cottony white to pinkish, irregularly branched and measuring upto 3 μ in diameter. In old cultures, the hyphae are more or less thickened. Study of various free hand sections from the lesions of infected fruits revealed the parasite to be intracellular.

On green fruits, the pustules resemble the cankers on the whole and vary in size from a pin-point to 2 mm. They are dark brown, subglobose, gregarious and contain numerous conidia.

Abundant conidia are produced both on the host tissue and in several culture media.

The dark, black and circular pycnidia on the culture media and fruits contain conidiophores and conidia. The conidia are typically 5-celled, oblong, clavate or elliptic-fusoid, erect, hardly constricted at septa, measuring 13-31 $\times$ 5-10 μ ; 3 median cells are guttulate, highly brownish, the central cell being the thickest and greatly bulged ; end cells are hyaline ; the apical conic or cylindric cell grows out into 3 hyaline, slender, elongated appendages ; the basal cell is obtuse, erect with a small pedicel. The measurements of 50 conidia, obtained from cultures grown on oat meal, gram meal, Richard's and lima bean agars, by a filar micrometer under artificial light-are given in table III.

TABLE III

Measurements of conidia of P. psidii *obtained from different media*

Medium	Length in μ		Width in μ	
	Range	Mean	Range	Mean
Oat meal agar	13-25	21	6-7	6
Gram meal agar	16-26	20	6-8	7
Richard's agar	20-28	24	6-10	7
Lima bean agar	16-31	23	5-9	7

It will be seen that larger conidia are produced on lima bean and Richard's agars as compared to those produced on gram meal and oat meal agars. The range of conidial measurement is 13-31 $\times$ 5-10 μ. Guba (1932) gives 21-35 $\times$ 6-8 μ as measurement of conidia, probably from the host tissue. Young conidia are more or less globular.

PENETRATION OF THE FUNGUS IN HOST TISSUE

Free hand sections, made from the lesions of the naturally and artificially infected fruits at various stages of development of the disease, showed that the

intracelluar mycelium was not only localised in the tissues of affected portions, but also spreading internally to other healthy portions as well. At the end of fifteen days, the fungus was observed to penetrate the tissue below the point of inoculation to the extent of 4-6 mm.

The fruiting bodies consisted of pycnidia formed at the end of 7-8 days, below the epidermis in the centre of the lesions. Each pycnidium consists of a stromatic layer from the surface of which are formed simple, erect, hyaline, continuous conidiophores packed closely together. The conidia are produced at the tips of conidiophores, and, with their accumulation, epidermis is ruptured.

PHYSIOLOGY OF THE FUNGUS

GROWTH ON VARIOUS CULTURE MEDIA. *Pestalotia psidii*, when grown on oat meal, gram meal, potato dextrose, green guava decoction and Richard's agars, to obtain comparative data on mycelial growth and the production of reproductive bodies, was found to grow vigorously on all except the green guava decoction agar, where only scanty vegetative growth was formed. On other media, the fungus produced profuse mycelial growth in concentric rings. The cottony white aerial mycelium was raised above twe surface, to a height of about 3-4 mm. in case of lima bean and gram meal agars, while it produced abundant but subaerial mycelium on oat meal agar.

The mycelial growth in all cases covered the entire surface of the plate in eight days after inoculation, but on Richard's agar, the growth though profuse was comparatively slow (Plate 1, fig. 4).

Sporulation was quick and abundant on Richard's and potato dextrose agars, slow on other media, and absent on green guava decoction agar.

GROWTH IN RELATION TO TEMPERATURE. The linear growth of the fungus and rate of sporulation in relation to temperature was studied in poured plates of Richard's agar incubated at the following temperatures : 0°, 5°, 15°, 26°, 30°, 32° and 36°C.

The tests were run in triplicates and the results in table IV represent the average growth and sporulation after 7 days.

TABLE IV

Growth and sporulation of P. psidii *at various temperatures*

Temperature in (°C)	Colony diameter in mm.	Sporulation
0	0.0	Nil
5	10.0	Slight
10	25.0	Slight
15	36.0	Abundant
26	75.0	Profuse
30	55.0	Moderate
32	15.0	Nil
36	0.0	Nil

P. psidii grew more vigorously at temperatures between 15-30°C, although it made good growth at 26°C and failed to grow above 32°C and below 5°C.

CARBON REQUIREMENTS OF THE FUNGUS. The carbon requirements of *P. psidii* were studied on modified Richard's agar (sucrose omitted) as a base to which various carbon compounds were added in the concentration of one per cent. Equal quantities of media were poured in petri dishes which were then inoculated in duplicate and incubated at 26°C. At the end of 7 days, measurements of colony diameter, observations on sporulation and growth characters were made and are presented in table V.

TABLE V

Colony diameter, sporulation and growth characters of P. psidii *on modified Richard's agar containing various carbon compounds*

Source of carbon	Growth characters	Sporulation	Colony diameter in mm.
1. Mannitol	Very good concentric rings. Mycelium aerial and cottony white.	Abundant	65.0
2. Dextrine	Good concentric rings. Mycelium aerial and cottony white.	,,	60.0
3. Sucrose	Very good. Mycelium aerial, buff white.	,,	75.0
4. Lactose	Good. Hyaline, radiating hyphae scattered all over, and thick whitish fluffy growth overlapping at certain areas. Mycelium subaerial.	Moderate	75.0
5. Maltose	Good concentric rings. Mycelium yellowish white.	Slight	40.0
6. Salicin	Good. Flat at sides and centrally raised. Serrated margin, brownish at sides and cottony white in centre.	Nil	35.0
7. Glycerol	Scanty, slightly radiating hyphae visible, hyaline.	,,	Scanty
8. Ripe guava decoction	Scanty, slightly radiating hyphae visible.	,,	,,
9. Modified Richard's agar (Control)	,,	,,	,,

Variation in growth rates was quite significant with different sources of carbon. The fungus grew profusely on media containing mannitol, dextrine and sucrose, fairly on maltose and salicin, and poorly on ripe guava decoction, glycerol and Richard's modified agars.

Best sporulation was obtained on media containing mannitol, dextrine and sucrose and slight or nil on maltose, lactose, glycerol, salicin, guava decoction and Richard's modified agars.

GROWTH IN RELATION TO NITROGEN UTILISATION. To study the nitrogen metabolism of *P. psidii*, modified Richard's agar (potassium nitrate omitted) was used as a base to which various compounds containing inorganic and organic nitrogen of the highest purity were added in the concentration of one per cent. At the end of 7 days, records on measurements of colony diameter, sporulation and growth characters were made and are given in table VI.

TABLE VI

Growth of P. psidii *on modified Richard's agar containing various nitrogen compounds*

Source of nitrogen	Sporulation	Colony diameter in mm.
1. Potassium nitrate	Profuse	80.0
2. Asparagin	,,	75.0
3. Ammonium tartrate	Abundant	60.0
4. Ammonium lactate	,,	60.0
5. Sodium nitrate	Moderate	75.0
6. Ammonium sulphate	Slight	45.0
7. Amyl nitrate	,,	70.0
8. Ammonium phosphate	Nil	65.0
9. Ammonium nitrate	,,	60.0
10. Modified Richard's agar (Control)	,,	75.0
Sodium nitrite	,,	Nil

With different sources of nitrogen, the growth characters of *P. psidii* remained practically constant. The growth in all cases was thick, fluffy, and raised usually with entire margin except in the case of ammonium sulphate, asparagin, and sodium nitrate where the margin was serrated. The fungus also produced subaerial mycelium.

Marked variations were, however, obtained with different sources of nitrogen with regard to growth as expressed by colony diameter and sporulation. While potassium nitrate, ammonium lactate and asparagin favoured the development of both the phases of the fungus, the modified Richard's ammonium nitrate, ammonium phosphate and amyl nitrate induced fair to good growth but no sporulation. Sodium nitrite inhibited both phases. These results are in perfect harmony with those obtained by Patel and Kulkarni (1949) with 7 fungi, 5 parasitic and 2 saprophytic.

INFLUENCE OF H-ION ON GROWTH. *P. psidii* grew in a wide range of H-ion concentration, optimum being3.9-4.9 as indicated by the dry weights of the fungus felt. The maximum growth was obtained at pH 4.9, indicating that the acidic reactions were more favourable to growth of the fungus than the alkaline ones. Fair to good growth was made at pH ranging from 2.9-6.6. It was also noted that the pH of the medium after 7 days of incubation changed from more acidic to less acidic.

STUDIES IN GERMINATION

EFFECTS OF TEMPERATURE ON SPORE GERMINATION. Hanging drop cultures were made with sterile water and incubated at various temperatures ranging from 5-36°C. Average of three readings taken at the end of 20 hours, in respect of per cent germination and length of germ tube, is given in table VII.

TABLE VII

Effect of temperature on per cent germination and length of germ tube of spores of P. psidii

Temperature (°C)	Per cent germination	Length of germ tube in μ
5	0.0	0.0
10	10.4	46.5
15	12.5	83.5
26	25.1	158.0
30	28.2	170.0
32	21.7	95.3
36	0.0	0.0

It will be observed that the germination of spores and the length of germ tube were greatly influenced by temperature. The spores of the fungus started germinating at 10°C and increased with rise in temperature upto 32°C. The same is true in the case of length of germ tube. This indicates that there

exists a definite and close correlation between the length of germ tube and the per cent germination.

INFLUENCE OF HOST TISSUE ON GERMINATION OF SPORES. Hanging drops of spore suspension in sterile water, green guava fruit decoction, unripe and ripe guava fruit tissues mixed separately in sterile water and sucrose solution, were incubated at 24-26°C. The results given in table VIII represent germination per cent made after six hours.

TABLE VIII

Effect of host tissue on germination of spores of P. psidii

Medium	Per cent germination
1. Sterile water	8.3
2. Green fruit decoction	66.9
3. Sterile water with green fruit tissue	91.7
4. Sterile water with ripe fruit tissue	40.0
5. 1% sucrose solution	19.6

Addition of green fruit tissue and its decoction gave greater stimulation to germination than the ripe fruit tissue.

OVER-SUMMERING OF THE PATHOGEN

Leaves of guava showing heavy natural infection were kept in glass vials, and kept hanging in the open under natural environmental conditions. Periodical germination tests were made of the spores, obtained from the leaves, for a period of six months, to ascertain the viability of spores during the severe summer months at Poona, where the temperature of 106°F was recorded during the period of experiments. The results are summarised in table IX.

TABLE IX

Viability of P. psidii *spores*

Date	Average per cent germination
2-1-49	62.3
2-2-49	40.4
2-4-49	37.4
2-6-49	25.2
2-7-49	9.1

These results show clearly that while the per cent germination of spores was retarded with the increase in temperature, they can survive the varying conditions and high temperature of Poona summer, and provide sufficient inoculum for reinfection and renewed activity, with the restoration of favourable conditions of temperature and humidity of monsoon season.

Indications that the period of viability of the fungus is determined by the specific host tissue affected have also been obtained. Longer period of viability can be obtained with spores obtained from fruit lesions than from leaf lesions, as the former pustules are better protected by a corky, impermeable, raised tissue.

DISCUSSION AND CONCLUSION

Though the disease is generally not of a serious nature, it causes considerable damage to green fruits in years of high humidity and rainfall. The fungus attacks the green fruits only in nature, and produces raised, circular pustules, characteristic of the disease. Inoculation experiments made at different stages of the fruit development confirm the observation recorded.

The formation of conidia in culture and their germination are typical and the studies, in the latter aspect, have shown that while normally conidia germinate sparingly in sterile water, addition of green fruit tissue to water greatly stimulates germination as expressed both by per cent germination and length of germ tube. Ripe tissue does not, however, appear to exert much influence; this important observation is in accord with general observations carried out in nature with regard to the stage of development of the fruit in relation to infection. Ripe fruit is much less susceptible to infection than green fruit. Of the several horticultural varieties of guava tried, all were susceptible except the Nasik variety, Lucknow 49 being very highly susceptible.

While the fungus grew best and produced abundant sporulation at 26°C, the optimum for germination of spores was slightly higher, *viz.*, 30°C, although the range is identical in both cases. Sporulation was greatly stimulated by the addition of sucrose and mannitol to the medium, but remained unaffected with ripe guava decoction; this is contrary to the results obtained for conidial germination.

Studies in over-summering of the fungus have conclusively proved its ability to withstand high and varying temperatures of Poona summer, even in the conidial stage, although longer periods of viability were obtained with conidial pustules on fruits than those on leaves, due probably to a better protective layer over the pustules in the case of the former.

SUMMARY

The guava canker caused by *P. psidii* was studied in detail for the first time.

In nature, the disease is found to occur mostly on green fruits and rarely on leaves. The symptoms of the disease have been described in detail.

The pathogenicity of the fungus has been proved on guava fruits. Several varieties of guava were noted to be susceptible to infection by the fungus. Lucknow 49 was highly susceptible while Nasik highly resistant.

The cardinal temperatures for growth of the fungus in potato dextrose agar were 5°, 26° and 32°C.

The cardinal temperatures for conidial germination were 10°, 30° and 32°C.

Sporulation was greatly stimulated by the addition of sucrose and mannitol to the medium, while it remained inhibited with guava decoction.

Green fruit tissue and decoction stimulated germination of spores over that in sterile water alone. Ripe fruit tissue and sucrose solution did not appear to exert much influence on germination.

The fungus remained viable in conidial stage under varying temperatures of Poona summer, ranging from 102° to 106°F, thus supplying sufficient inoculum for re-infection in the monsoon.

The authors wish to thank Dr. B. B. Mundkur for helpful suggestions while writing this paper.

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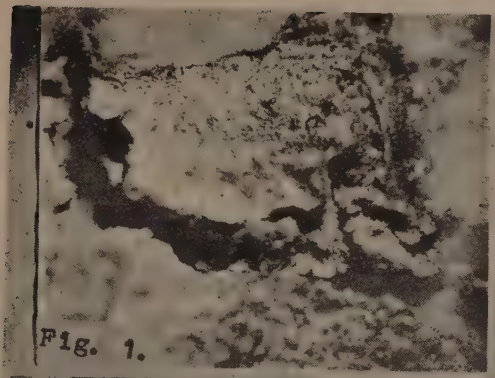

Fig. 1.

Fig. 2

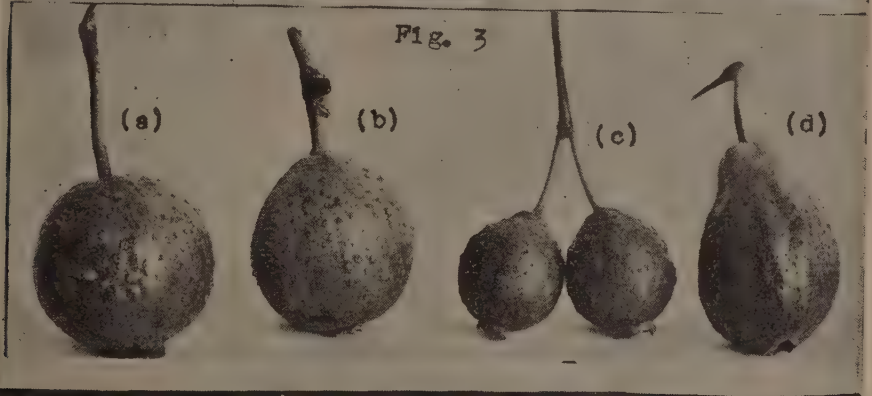

Fig. 3

Fig. 4

NOTES ON SOME SPORE GERMINATION & MOUNTING TECHNIQUES

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(Accepted for publication September 15, 1950)

(A)

In earlier papers, Thirumalachar (1940) and Thirumalachar & Dickson (1947) described a method for germinating and staining teliospores of rusts or chlamydospores of smuts. The spore material to be germinated was fixed to the slide by alternate wetting and drying of the spore mounts. The spore material thus fixed to the slide was inverted over a water surface supported by racks, and wet paper toweling was placed on the backside of the slides to help in condensing films of water on the inner side. Spore germinations in several smut fungi, such as *Ustilago striiformis* and *Urocystis agropyri*, which were previously known to be difficult to germinate or not germinated at all, were secured by following this technique.

In place of glass dishes and racks that were needed to set up the germination experiments, an improvised germination chamber was found very useful, a short description of which is given here. Card board boxes of photographic plate holders (quarter plate size) or card board containers of filter papers (usually measuring 13×13 cms.) were found to be very suitable for the purpose. The slides were supported on two card board strips (2 cms. high) on either side. The whole box and lid were immersed in molten paraffin, which not only rendered the chamber water proof, but also held the two strips in position on hardening.

Before placing the slides for germination, a thick wad of cotton thoroughly moistened in water was placed on the floor of the chamber between the card board strips. After the slides with the spore mounts were inverted over the moist cotton, a thick band of moist paper toweling or moist cotton wad was placed on the backside of the slides (Fig. 1). The lid of the chamber was partially closed to permit evaporation current. Within an hour, the deposition of fine films of water on spore mounts became apparent.

The slides were examined at different intervals, and when sufficient number of spore germinations had taken place, they were fixed and stained as in the case of paraffin sections. Since the spore material was made to adhere to the slides before placing for germination, they were not dislodged during staining procedures.

(B)

Recently, Dade and Waller (1949) described a sealing wax mixture for making mounts in lactophenol permanent. It consisted of refined bees-wax and gum dammar mixed in equal quantities melted on a gentle Bunsen flame. The wax is satisfactory as far its qualities like toughness, adhesiveness to a glass surface, convenience in handling and standing against lactophenol are concerned. But, however, it was found unsuitable to work with during summer seasons. The wax ring invariably came off with the cover glass while manipulating with the objectives, since the sealing became soft and sticky and was still of temporary nature. This diffculty was over-come by further manipulation which rendered the sealing more permanent.

After sealing with the dammar bees-wax, a fine ring of canada balsam, of medium consistency in xylol, was drawn over the wax, and the slide kept on a hot plate at 60°C for about half an hour. The wax melted incorporating the canada balsam and, on cooling, formed a uniform perfect seal, which was quite hard, and translucent without fogging the mountant fluid.

The same sealing technique has been used with very good results for making permanent iron aceto-carmine squash preparations of pollen mother cells and root tips, and mounts of algae and prothalli of ferns. The sealing was done first with dammar bees-wax mixture and later with canada balsam as described above.

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ON THE OCCURRENCE OF A GENUS OF FUNGI IMPERFECTI BLASTOTRICHUM CORDA IN INDIA

DAYA NAND PANT

(Accepted for publication October 1, 1950)

WHILE studying the fungi of Lucknow, the author isolated a superficial fungus from fruits of *parwal* (*Trichosanthes dioica* Roxb.). There was a mouldy growth on the fruits which, on culturing, showed white mycelial growth, and, on examination, appeared to be a species of *Blastotrichum* Corda. Review of literature indicates that no species of *Blastotrichum* has so far been reported in India. This fungus was isolated from fruits purchased from vegetable market which were quite fresh and green, which gives the suspicion that the fungus is a parasitic one. Further search, this year, for infected fruits from vegetable market proved unsuccessful. It is intended to make further search and study the disease in *parwal* fields next season.

There was a minute mouldy growth on the outside of some fruits. Isolations from the outer and inner portions gave white mycelial growth in standard synthetic agar medium. Within two to three days the mycelial growth was copious. There were prostrate vegetative hyphae and erect aerial, branching conidiophores bearing the conidia, there being no difference of shape, size or colour between the vegetative hyphae and conidiophores. Both are hyaline, and poorly branched, being 1.5-2.5 μ wide. The conidia are clavate, 6-7 μ thick and 20-30 μ long, having 3-5 septa, the vast majority being with three septa and those with five septa being very rare.

The species under study is very similar to the published description of *B. floccossum* Hartz in conidial and hyphal shape and colour, but the size of conidia and the width of hyphae differ considerably from *B. floccossum*. The present fungus is, therefore, presented as new with the specific epithet *Chowdhuryi*, named in honour of Prof. H.P. Chowdhury of Lucknow University.

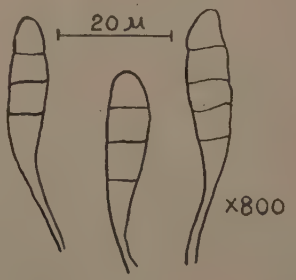

Fig.

Blastotrichum Chowdhuryi Pant. Mycelium mouldy, hyphae and conidiophores hyaline, branched irregularly, non-septate, 1.5-2.5 μ wide. Conidia clavate, hyaline, rounded on both sides, 3-5 septate, 20-30 $\mu \times$ 6-7 μ.

In fruits of *Trichosanthes dioica* Roxb. collected by the author during August, 1948.

Type in Botany Department, Lucknow University, and I. A. R. I. New Delhi.

Blastotrichum Chowdhuryi Pant. Caespitulis mucedineis, hyphis et conidiophoris hyalinis, ramosis irregularibus, non-septatis, 1.5-2.5 μ diam. Conidiis clavati, hyalinis, utrinque rotundatis, 3-5 septatis, 20-30 $\mu \times$ 6-7 μ.
Hab. in fructus *Trichosanthes dioica* Roxb.

SUMMARY

(1) Genus *Blastorichum* Corda has been reported from India.

(2) A new species *Blastotrichum Chowdhuryi* sp. nov. has been described.

Thanks are due to Prof. H. P. Chowdhury, Head of the Botany Department, Lucknow University, for his interest and constant guidance throughout this work and, to the authorities of the Lucknow University, for the award of a grant-in-aid for some months. Thanks are also due to Dr. B. B. Mundkur and Dr. R. S. Vasudeva for some information regarding this work.

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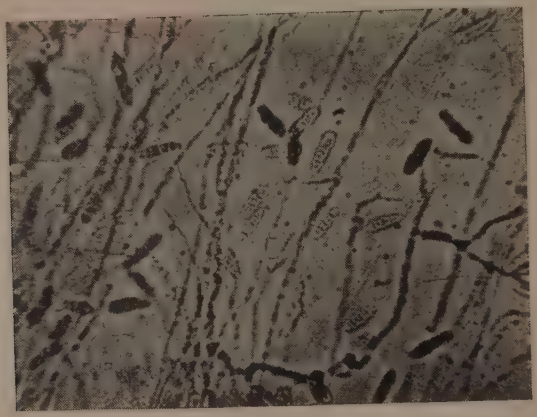
Explanation of Plate

Photomicrograph of *Blastotrichum Chowdhuryi* showing conidiophores and conidia. × 300.



FURTHER STUDIES ON *CUCUMIS* VIRUS 2C

S.P. Raychaudhuri, S.B. Lal and R.S. Vasudeva

(Accepted for publication December 30, 1950)

Introductory

A new strain of *Cucumis* virus 2 responsible for mosaic disease of bottlegourd (*Lagenaria leucantha* (Duch.) Rusby) was described by Vasudeva *et al* (1949) and designated 2C. Properties of this strain of the virus have been further studied and are now reported.

The experimental work was conducted with the initial inoculum multiplied on young bottlegourd plants in the insect-proof house. Inoculations were normally done with standard extract, prepared by crushing the infected leaves to every gram of which 1 c.c. of sterilised distilled water was added. Throughout the course of these experiments actively growing plants, about 3 weeks old, were used for inoculation purposes, as a previous infectivity test conducted with plants of different ages, varying from 3 to 52 days, showed 100 per cent infection in plants 17 to 38 days old. Both old and young plants of ages 52 and 3 days in the series showed an infection percentage of 50 and 60 respectively.

Experimental

A. pH-stability of the virus. The method followed for the determination of pH stability of the virus was essentially the same as described by Samuel, Best & Bald (1935). The standard extract of the virus used in these series of experiments was first stored for about 24 hours at room temperature (28°-30°C.), and then centrifuged at 3,500 r.p.m for 20 minutes in a 'Wifug' type centrifuge, which yielded a clear brownish liquid after removal of the extraneous matter. A stock composite buffer solution 0.0533 M with respect to each of boric acid, potassium dihydrogen phosphate and potassium hydrogen phthalate, was prepared and used throughout, except where otherwise mentioned. Aliquots of the buffer were adjusted between pH values 1 and 10, at intervals of approximately one pH unit, by the addition of appropriate amounts of 0.2 M hydrochloric acid or 0.2 M sodium hydroxide and distilled water, to give a final solution of the desired pH value and being 0.04 M with respect to the total buffering substance.

Hydrogen-ion concentration of each inoculum was determined with the help of glass electrode immediately after preparation, as also before inoculation, throughout the course of the experiment. The accuracy of the measurement was tested by comparing the pH of two solutions obtained both by the glass electrode and the hydrogen electrode. No appreciable change in the pH values of the inoculum was observed over the time ranges employed.

One c.c. of the centrifuged virus extract was added to 9 c.c. of different adjusted buffer solutions with vigorous shaking. Immediate inoculations were

made to test the effect of H-ion concentration on infectivity. With a view to study the temperature-activity and the time-activity relationships, simultaneously with the pH relationship of the virus, the inocula adjusted to different H-ion concentrations were divided into three lots. Two lots were stored, one at 7°C and the other at room temperature (28-30°C). The third lot was incubated at 7°C for 7 days and then transferred to room temperature. The infectivity of each was tested after storage for a maximum period of fifteen days. A blank experiment was conducted in the same way after adjusting the H-ion concentration of the extract, collected from healthy leaves, to 3.0, 4.15, 5.36, 6.7 and 8.12. All the test plants in this experiment remained healthy.

The data showing the infectivity of the virus at various H-ion concentrations are set out in Table I.

The data show that the pH range of maximum infectivity is between pH 1.88 and pH 9.96. The pH stability range, on the other hand, is dependent on temperature and the period of storage. The virus is active between pH 1.88 and pH 9.96 till 24 hours both at 7°C and room temperature, while after 7 days the virus at room temperature is inactivated at pH 1.88, but retains its activity at 7°C. After 15 days, the virus is active between pH 2.88 and 9.96 at 7°C, while between pH range 3.04 and 9.96 at room temperature. The virus stored for 7 days at 7°C and then for 8 days at room temperature showed activity over pH 2.28 to pH 9.96. It is indicated that the pH range for maximum infectivity of the virus is greater than the range of maximum stability, as also the virus has wide range of pH stability.

Since there are differences in the time and temperature co-ordinated with pH stability ranges of viruses already described by various investigators, exact comparison is difficult. The pH stability range of the virus under study, however, appears to differ considerably from cucumber mosaic virus (Stanley, 1935) which has a much narrower range of pH stability *viz.*, 5.0 to 7.0, whereas its stability favourably compares to some other viruses like tobacco mosaic (Stanley, 1945), tomato bushy stunt (Bawden & Pirie 1938) and tobacco necrosis (Price, 1938), the stability of which lies between pH 1.5-9.2, 1.5-9.5 and 3.1-9.7, respectively. It is evident from the results obtained by previous workers that the pH stability range is reduced with the time of storage. Thus, for tobacco ringspot virus (Stanley, 1939), the range is pH 4.0-9.5 and 6.0-9.0 in two days and five days, respectively, while for alfalfa mosaic virus (Ross, 1941) it is pH 5.0-8.0 after 20 hours but pH 5.5-7.7 after 120 hours, and for bottlegourd mosaic virus, described herein, it is pH 1.88-9.96 after 7 days at 7°C and pH 2.28-9.96 and 3.04-9.96 after 15 days at 7°C and room temperature, respectively. It is also shown by Price (1945), in case of southern bean mosaic virus, that high temperature reduces the pH stability range so that it is between pH 4.0-8.0 at 3°C, while it is between pH 5.0 and 6.7 at 27°C.

B. REACTION OF THE VIRUS TO CHEMICALS. Fresh stock of standard extract of the virus was prepared and centrifuged to obtain clear liquid as before. Known volumes of standard virus extracts were treated with different chemicals to study their effect on the virus activity.

Young bottlegourd plants were inoculated with the virus extracts previously treated with different chemicals soon after the treatment, at intervals of

TABLE I

Effect of H-ion concentration on the activity of the virus stored for different periods at different temperatures

pH of inoculum	Immediate inoculation		Room temperature (28°-30°C.) 1 day		7 days		15 days		7°C. 1 day		7 days		15 days		From 7°C. to Room Temp.	
	No. of plants		No. of plants		No. of plants		No. of plants		No. of plants		No. of plants		No. of plants		No. of plants	
	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected
1.88	6	6	6	3	6	0	6	0	6	6	6	6	6	0	6	0
2.28	6	6	6	3	6	4	6	0	6	6	6	6	6	4	6	4
3.04	6	6	6	3	6	3	6	2	6	5	6	5	6	4	6	4
4.45	6	5	6	4	6	4	6	3	6	5	6	5	6	4	6	4
5.40	6	5	6	4	6	4	6	4	6	4	6	4	6	4	6	3
6.10	6	5	6	5	6	6	6	3	6	4	6	5	6	4	6	4
7.12	6	6	6	4	6	3	6	3	6	5	6	4	6	4	6	3
7.98	6	6	6	4	6	2	6	3	6	4	6	4	6	5	6	5
8.63	6	5	6	3	6	2	6	2	6	4	6	2	6	3	6	3
9.96	6	4	6	3	6	2	6	2	6	4	6	3	6	2	6	2

one hour and 24 hours. After storage of the treated material for 24 hours, two sets of inoculations were done. In one case the preserved material was used as such, but in the other case the treated material was diluted with distilled water in the proportion of 1: 100, in order to reduce the toxic effect of the chemical under test, if any, on the plant. The material in these tests was always stored at 7°C. The results of infection tests with virus extracts treated with different chemicals are given in Table II.

TABLE II

Reaction of the virus to chemicals

Chemicals tested	Immediate inoculation		Inoculation after 1 hour		Inoculation after 24 hours		Inoculation after 24 hours after being diluted to 1 : 100	
	No. of plants		No. of plants		No. of plants		No. of plants	
	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected
Nicotine sulphate (Commercial)								
1%	6	6	6	5	6	5	6	5
2%	6	5	6	5	6	4	6	5
50%	6	0	6	0	6	0	6	3
Copper sulphate								
0.5%	6	0	6	0	6	0	6	6
1%	6	0	6	0	6	0	6	4
2%	6	0	6	0	6	0	6	3
Hydrogen peroxide								
40% of 30 vols.	6	6	6	4	6	6	4	3
50% „ „	6	5	6	4	6	0	4	0
Potassium permanganate								
0.5%	6	5	6	4	6	6	6	3
1%	6	3	6	3	6	0	6	0
2%	6	3	6	0	6	0	6	0
Mercuric chloride								
0.1%	6	5	6	6	6	4	6	4
0.25%	6	3	6	4	6	3	6	4
0.5%	6	0	6	0	6	0	6	0
Alcohol								
20%	3	3	3	3	3	3	—	—
30%	3	2	3	3	3	2	—	—
40%	3	3	3	2	3	3	—	—
50%	3	3	3	3	3	3	—	—

TABLE II (*Continued*)

Chemicals tested	Immediate inoculation		Inoculation after 1 hour		Inoculation after 24 hours		Inoculation after 24 hours after being diluted to 1 : 100	
	No. of plants		No. of plants		No. of plants		No. of plants	
	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected
Formalin								
0.5%	6	4	6	4	6	0	6	0
1%	6	4	6	0	0	0	6	0
2%	6	0	6	0	6	0	6	0
Glycerine								
30%	6	6	6	4	6	4	6	6
40%	6	5	6	5	6	5	6	5
50%	6	5	6	5	6	5	6	4
Carbolic acid								
0.05%	6	5	6	4	6	3	6	3
0.5%	6	5	6	5	6	4	6	4
1%	6	6	6	6	6	0	6	0
Silver nitrate								
0.5%	6	4	6	3	6	4	4	2
1%	6	4	6	4	6	0	4	0
Acetone								
40%	6	6	6	4	6	4	4	2
50%	6	4	6	3	6	4	4	3

The results indicate that 50% alcohol, 0.5% silver nitrate, 2% nicotine sulphate, 0.25% mercuric chloride, 40% hydrogen peroxide, 0.5% carbolic acid, 50% glycerine, 0.5% potassium permanganate and 50% acetone do not inactivate the virus even after storage for 24 hours, so that the chemicals neither affect the plant nor the virus. Successful infections immediately after treatment with chemical, but no infections after storage, indicate that the chemicals have no effect on the plant but inactivate the virus, so that 0.5% hydrogen peroxide, 1% potassium permanganate, 1% carbolic acid and 1% silver nitrate have no effect on the virus after one hour, but render it innocuous after 24 hours. The virus is inactivated by 1% formalin, and 2% potassium permanganate in one hour. With 50% nicotine sulphate, 2% formalin, 0.5% copper sulphate and mercuric chloride, the plants fail to take infection when inoculated immediately, after one hour, and 24 hours, indicating that either the inactivation of the virus or toxicity of the chemical to the plant, or both, may be responsible for failure of the plants to take infection. The leaves of healthy plants when rubbed with these chemicals showed that these have direct toxic effect on them, but the whole plant remains unaffected.

The virus extracts treated with 50% nicotine sulphate and 0.5%, 1% and 2% copper sulphate, after storage for 24 hours and dilution in the proportion of 1 : 100, when tested on plants, gave successful infection, which indicates that, probably, the loss of infection is due to their inhibitory action on the host plant. The plants do not get infected after inoculation with the virus treated previously with 2% formalin and 0.5% mercuric chloride, even on diluting the inoculum, which may be due to the inactivation of the virus or due to inhibitory effect of the chemical, even after dilution, on the host. On immediate and after one hour inoculations, 0.5% formalin, 50% hydrogen peroxide, 1% carbolic acid, potassium permanganate and silver nitrate do not have any inhibitory effect on the test plants which took infection, but on inoculation after 24 hours even after diluting the inoculum the plants failed to take infection. This shows that the chemicals cause the virus to inactivate in 24 hours. Further, the virus extract when treated with 1% formalin and 2% potassium permanganate and inoculated immediately produced infection on test plants, but failed to do so when inoculated after one hour which indicates that these chemicals inactivate the virus even after one hour.

Doolittle (1920) observed that *Cucumis* virus 1 (Smith, 1937) is inactivated after one hour by treatment with 0.5% formaldehyde and copper sulphate, 50% alcohol, and mercuric chloride 1 : 2,000. *Cucumis* virus 2 (Bewley, 1926) and its strain 2B (Capoor & Verma, 1948) withstand treatment with 50% and 95% alcohol, respectively, for 24 hours. Vasudeva and Lal (1943) reported the inactivation of *Cucumis* virus 3 by acetone and 50% alcohol, as well as by mercuric chloride, 1 : 5,000, in 10 minutes.

Besides the chemical inactivation of the virus or the inhibitory action by the chemical on the plant, it is also observed that the reaction due to treatment with the chemical is directly proportional to the concentration of the chemical and the time of action. Thus, 50% nicotine sulphate, 1% and 2% formalin, 50% hydrogen peroxide, 1% and 2% potassium permanganate, 1% carbolic acid, 1% silver nitrate and 0.5% mercuric chloride have more pronounced effect on the activity of the virus than that due to the same chemicals at lower concentrations. When treated with 1% formalin, 2% potassium permanganate, 1% carbolic acid, 1% silver nitrate, 1% potassium permanganate, and 50% hydrogen peroxide, the virus is capable of infecting bottle-gourd plants on immediate inoculation, but not after one hour and 24 hours storage, respectively.

C. ULTRAVIOLET IRRIDIATION OF THE VIRUS. The effect of radiant energy on the activity of the virus was tested by inoculating young bottle-gourd plants with the standard extract, which had been previously exposed to ultraviolet light in the Divison of Chemistry. For this purpose, an Alpine Sun lamp, Model IX, in which dosage can be calculated in the minimal units* was used. When the material is exposed for a period of 45 seconds, at a distance of 24 inches from the arc tube, it receives a minimal unit of dosage. The range of the spectrum obtained from the lamp, as taken on an Intermediate Quartz Spectrograph using an Ilford special rapid plate, was found to be from 2,200°A to 6,000°A. For every exposure, 5 c.c. of the clarified standard extract were pipetted out in each of

*A minimal unit of dosage of exposure to ultraviolet irradiation is 5,22,000 ergs per sq. cm. of the Erythema producing ultraviolet rays.

the three petri dishes of 7 cm. diameter and exposed, at a distance of 24 inches, for varying periods, to the ultraviolet lamp running on 220 volts D. C. Controls were maintained with standard extract which had not been exposed to light. The results of the experiment are set out in table III.

TABLE III

Effect of ultraviolet radiation on the activity of the virus

Period of exposure (Minutes)	Dose in Minimal units	*Number of plants* inoculated	infected
5	6.66	6	6
15	20.00	6	6
60	80.00	6	6
75	100.00	6	6
90	120.00	6	4
105	140.00	6	1
120	160.00	6	0
180	240.00	6	0
Unexposed Control.	—	6	6

The data given above show that the activity of the bottle-gourd mosaic virus in standard extract is not adversely affected when exposed for 75 minutes (dose of 100.00 minimal units), but begins to fall after exposure for 90 minutes (120.00 m. u. d.), and is rendered innocuous after 120 minutes (160.00 m. u. d.)

Ultraviolet light has been found to be lethal for tobacco mosaic virus, and a period of 15 seconds to one or more hours has been estimated by several investigators to be the time required for inactivation, depending on the freedom of the virus preparation from extraneous material and the intensity of irradiation. Smith (1926) observed the inactivation of filtered juice by an exposure of 30 minutes to the abiotic rays, while Mulvania (1926) reported that tobacco mosaic virus was killed in one hour when exposed to ultraviolet light from a Cooper-Hewett Mercury vapour lamp at a distance of 6 inches. Hollaender and Duggar (1936) observed the inactivation of tobacco mosaic virus by exposure to ultraviolet rays at relatively shorter wave lengths. Price and Gowen (1937) studied the effect of ultraviolet radiation on inactivation of tobacco mosaic virus, either wet or dried, purified or in natural plant juice. The rate of inactivation was greatest with purified virus and least when it contained extraneous matter to absorb the energy. The authors are much indebted to Dr. C. Dakshinamurthy for his valuable help in connection with ultraviolet radiation experiments and to Dr. B. B. Roy for kind cooperation.

SUMMARY

The pH infectivity of *Cucumis* virus 2C lies between 1.88 and 9.96. The pH stability, however, is affected by temperature and the period of storage.

The virus in standard extract is resistant to 1-2% nicotine sulphate, 40% hydrogen peroxide, 0.5% potassium permanganate, carbolic acid and silver nitrate, 0.1-0.25% mercuric chloride, 50% alcohol, glycerine and acetone. The virus is inactivated in 24 hours by treatment with 50% hydrogen peroxide, 1% potassium permanganate, carbolic acid and silver nitrate and also 0.5% formalin, while 2% potassium permanganate and 1% formalin render the virus innocuous in one hour.

The activity of the virus when exposed to ultraviolet light begins to fall after 90 minutes and the virus rendered innocuous after 120 minutes.

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STUDIES ON CLOVER RUST UROMYCES TRIFOLII (Hedw. f.) Lev., IN INDIA

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(Accepted for publication December 31, 1950)

CLOVER, *Trifolium resupinatum* L., is one of the most important fodder crops cultivated in India, Europe and America. It is affected by an autoecious, longcycle rust, *Uromyces trifolii*, which destroys the leaves, thereby reducing its fodder value to a considerable extent. Although the rust had been reported in 1908 by Butler on *Trifolium pratense*, and in 1915 by Shaw on *T. resupinatum*, it had not been studied in detail in India or elsewhere. Since a thorough understanding of the life history of the rust, its host range and mode of perpetuation is necessary before methods of control could be devised, the present work was undertaken.

Clover is sown in October-November and harvested in April. The rust has been observed to appear in February. The aecial stage appears first and is followed by the uredial and telial stages in March when the plants are mature. Teliospores and urediospores are formed in the same sorus.

There is no information on the factors governing the dormancy, viability and germination of teliospores of this rust. Since this information is necessary to determine the role of teliospores in the annual rust oubreaks, infected leaves of *Trifolium resupinatum* were collected from the field in April, and stored in sealed glass tubes at 8°-10° C. A part of this material was exposed to natural conditions and the rest kept in the laboratory at Delhi. Teliospores from each of these portions were tested for germination at 12°-18° C and 20°-22° C after every fortnight from the time of collection, according to the method described by Prasada[1]. No germination was, however, observed before November, *i.e.*, before seven months of their formation. It shows that the teliospores of this rust require a resting period.

When the resting period of the teliospores had expired, experiments were conducted to determine the precise temperature that would permit their germination. For this purpose, infected leaves taken from the three portions stored at different places, as described above, were separately soaked in water overnight, and teliospores were kept for germination at different temperatures between 3° and 35°C. The best germination was observed between 12° and 22°C, a few spores germinated at 6°-10°C and at 25°C, but no germination was observed at 3°-5°C and at 28°C, and above. All germination tests were, therefore, made

The authors are grateful to the Head of the Division, for facilities and helpful suggestions.

1. Prasada R. (1948) Studies on the formation and germination of teliospores of rusts. *Indian Phytopathology* **1** : 119-126

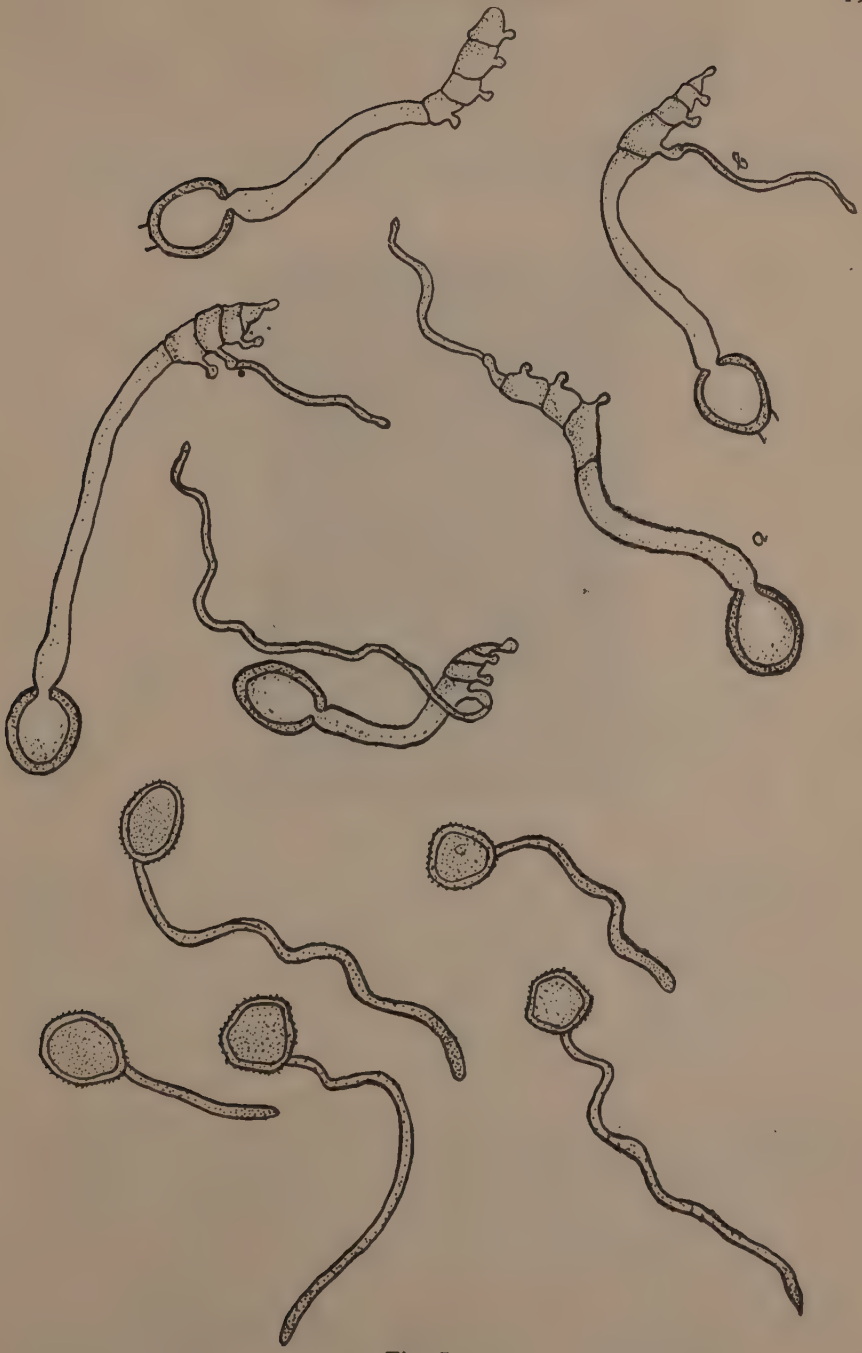

Fig. I

at 12°-22°C. At this favourable range of temperature, alternate drying and wetting of the host leaves in water, for two days of each operation, enhanced the percentage of germination. It was also found that exposure to natural conditions, subsequent to their formation, does not destroy the viability of teliospores. On germination, the unicelled teliospore produces a stout four-celled promycelium, each cell of which gives rise to a sporidium on a short sterigma (Fig. I a). The sporidia germinate bv means of a thin and long germ tube (Fig. I b).

Fig. II

Inoculations were made on clover plants with germinating teliospores. The technique followed was similar to the one described by Prasada[2]. Inoculations during December and January did not produce infection but positive results were obtained in February. Pycnia appeared in groups after 12 days, followed by aecia in 6 days. Young leaves, as well as old, got infected and produced aecia in abundance. Aecia with cup-shaped peridia are in groups on any tissue (Fig. II). Inoculations during the last week of March and April were unsuccessful because of unfavourable prevailing temperature.

The aeciospores are globoid, hyaline and finely verrucose, 13-16 × 15-20 μ, wall, nearly or quite colourless, about 1 μ thick. They germinate between 7°-30°C, irrespective of light or darkness and remain viable for 4 weeks when stored at 3°-5°C, for 13 days at 15°-18°C, for 6 days at 30°C, and for 48 hours at 38°C. When *Trifolium alexandrianum*, *T. minus*, *T. subterraneum*, *Medicago sativa*

2. Prasada R. (1948) Studies in Linseed Rust in India. *Indian Phytopathology* 1 : 1-18

and *T. resupinatum* were inoculated with aeciospores, only *T. resupinatum* got infected resulting in the formation of urediospores in 7-10 days.

Both sides of the leaf and petiole are infected. The uredia are small, scattered, amphigenous and light brown, sub-epidermal to erumpent. Urediospores are globoid or broadly ellipsoid, 18-22 × 20-25 μ, wall light cinnamon-brown, echinulate with 3-5 scattered pores.

Fresh urediospores germinate between 6° and 28°C with optimum at 20°-22°C. At the optimum temperature, the spores germinate in two hours in a moist chamber irrespective of light or darkness. Stored dry at 8°-10°C they remained viable for ten months, at 25°-28°C for 30 days, at 30°-32°C for 23 days, at 35°-38°C for 17 days, at 40°-42°C for 7 days and at 60°C for 48 hours.

A culture of this rust could not be maintained at Delhi during summer (April to October), in spite of repeated attempts with the urediospores stored at 8°-10°C. This was due to the fact that the urediospores do not germinate above 30°C.

Out of *Trifolium minus*, *T. repens*, *T. fragiferum*, *T. subterraneum*. *T. glomeratum*, *T. alexandrianum* and *T. resupinatum*, only *T. glomeratum* and *T. resupinatum* are susceptible to the urediospores of this rust.

DISCUSSION

There is no information on the perpetuation of this rust from one year to the next. The crop is harvested in April, and, from that time till its appearance in February, the course of its life history is obscure. The reaction of urediospores to temperatures above 40°C and the fact that cultures could not be maintained after April, show that oversummering of this rust in the uredial stage is not ordinarily possible. The aeciospores are similarly killed in six days at 30°C.

It has been shown that teliospores collected from the crop and exposed to natural conditions, as well as stored in the room, remain dormant for a period of seven months, retain their viability through the summer, and germinate to cause fresh infections during January-February, when suitable temperature (12°-22°C) and moisture are both available at the same time. These teliospores may come from plant debris of previous year's crop or from straw that may be stored with the seed. Burning the plant debris after harvest and use of clean seed should, therefore, control this rust to a considerable extent.

SUMMARY

The rust of clover, *Uromyces trifolii* appears in February. The aecial stage comes up first, and the uredial and telial stages are formed in March and April, respectively. Urediospores are the repeating spores.

The teliospores remain viable through the summer and germinate at 6°-25°C after a resting period of seven months. Longer storage and alternate drying and wetting hasten their germination.

Pycnia and aecia are obtained by inoculations with germinating teliospores in February and March.

Since the rust is unable to oversummer in the uredial stage, the teliospores which remain viable are responsible for fresh outbreaks in nature.

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COMPARATIVE STUDIES OF CERTAIN ISOLATES OF COLLETOTRICHUM FALCATUM Went

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(Accepted for publication December 31, 1950)

Introductory

THE most serious disease of sugarcane in India is, undoubtedly, red-rot caused by the fungus *Colletotrichum falcatum* Went. In the years 1938-41, the disease played such havoc with the sugarcane crop in India that it resulted almost in complete devastation of thousands of cane fields of the predominant commercial variety Co. 213 in eastern United Provinces and northern Bihar. Both the cultivator and the factory were very hard hit, so much so that the 'crush' of the sugar factories in the affected areas was reduced to one-third of the normal amount during 1938-39, and one-half in 1939-40.

In seeking the cause of this sudden failure of Co. 213, a detailed survey of the red-rot epidemic areas of northern India was undertaken by the senior author which revealed the presence of two strains, one light and one dark, distinguished by the colour and texture of the fungal colonies *in vitro* (Chona, 1940). Prior to this, all the isolates of *Colletotrichum falcatum* in India were, as judged by the information available, of a darker type with sparse sporulation. It was interesting to observe, therefore, that a light type of *Colletotrichum falcatum* with abundant sporulation predominated the epidemic areas, and that the new type was much more virulent than the old dark type. Chona (1942) further obtained several intermediate types *i.e.*, light coloured isolates with a tendency towards the looser, more floccose texture and lighter colour of the light type. Rafay and Padmanabhan (1941), in a survey of northern Bihar, also got the light and dark types, which they called A and B, and, in addition, an intermediate one which they termed as C.

These results are similar to those obtained by Abbott (1938) in the United States. In 1929-31, unusually severe losses were sustained from red-rot in Louisiana through the sudden failure of one of the leading commercial varieties P.O.J. 213. Isolations made from the cane varieties grown in Louisiana and southern Florida, showed that the fungus *Colletotrichum falcatum* consisted of two morphological types. On the basis of colour of the mycelium, they were termed light and dark types, respectively. Isolates intermediate in character between the two types were also obtained.

Ramakrishnan (1941) has studied the physiology of *Colletotrichum falcatum* (dark type). Lewton-Brain (1908), Edgerton and Moreland (1920), Sartoris (1929), Tims and Edgerton (1932) and Abbott (1938) also carried out cultural work with this fungus. Little attempt, however, seems to have been made in India to make a comparative study of the cultural and morphological characters

of different isolates and their pathogenicity. The present investigations were, therefore, undertaken for a better understanding of the parasitism and physiology of some typical isolates of *Colletotrichum falcatum.*

MATERIAL AND METHODS

Colletotrichum falcatum isolates 3, 29 and 78, obtained from red-rot affected canes from different localities and varieties, were selected for these studies as each represented a class by itself. Isolate 3 is a dark type with sparse sporulation and is weakly parasitic ; isolate 78, a light type with abundant sporulation, is highly virulent ; and isolate 29, a light coloured and freely sporing type, is intermediate to both 3 and 78 in virulence.

At the beginning of the work, purity of the three isolates was ensured by making a single-spore culture of each, following the method of Rawlins (1933).

The following media were used for comparison of cultural characters : Potato-dextrose agar (peeled and sliced potatoes, 200 g. ; dextrose, 20.0 g. ; agar agar, 20.0 g. ; distilled water, 1 litre), Oatmeal agar (oatmeal, 50.0 g. ; agar agar, 20.0 g. ; distilled water, 1 litre), Richards' solution agar (KNO_3, 10.0 g. ; KH_2PO_4, 5.0 g. ; $MgSO_4$, 2.5 g. ; saccharose, 30.0 g. ; agar agar, 20.0 g. ; and distilled water, 1 litre) ; and 'Cane-leaf decoction' as used by Abbott (1938).

For virulence tests, mature canes of the susceptible variety Co. 213 were selected, which were of the same age and size, usually about 70 inches in length and 1 inch in diameter. These were stripped and washed thoroughly with water. The middle internode was surface sterilized with rectified spirit and a hole was punched in its centre with a sterilized cork borer upto about two-third the thickness of the cane. The inoculum, consisting of young vigorously growing cultures, was introduced into the hole and the plug replaced in position. The point of inoculation was wrapped with a strip of paper and tied tightly with string to keep the plug in position. The inoculated canes were kept in a moist sand bed at the temperature of 30°-32° C. After about four weeks of their inoculation, they were split open longitudinally, and linear spread of the attack was measured. Proper controls were maintained.

GROWTH ON CULTURE MEDIA

The growth of each isolate was studied on potato dextrose agar, Richards' solution agar, oatmeal agar and cane-leaf decoction. Four Petri plates of each of the four media were inoculated with isolates 3, 29 and 78, and incubated for eight days at 30° C, at the end of which the diameter of the colonies along two directions, at right angles to each other, was measured. The average mean radial growth and the colony characters for the three isolates are presented in table I.

TABLE I

Growth characters of isolates 3, 29, and 78 on different media

Isolate	Mean radial growth in cms.				Nature of growth
	Oat-meal	Cane-leaf	Richards' agar	P.D.A.	
3	10.0	8.0	6.8	6.9	Good, dark gray mealy aerial growth, no pink spore masses, zonation absent, black stromatoid bodies formed.
29	10.0	8.2	7.0	7.0	Good, gray fluffy aerial growth, with very few pink spore masses, zonation partial, black stromatoid bodies formed.
78	10.0	8.3	7.1	7.1	Thin cottony growth, grayish white, numerous pink slimy masses of spores, zonation complete, stromatoid bodies absent.

It will be seen that the three isolates do not show any appreciable difference in their rate of growth on the four media. It has, however, been shown that oatmeal agar and cane-leaf decoction are relatively better suited for their growth than the other two media. Abbott (1938) and Ramkrishnan (1941) had also found that oatmeal agar was very good for general cultural work with *Colletotrichum falcatum*. Whenever a clearer medium was required, cane-leaf decoction was successfully used.

TEMPERATURE RELATIONSHIP

The effect of temperature on the rate of growth of the three isolates was studied. Petri plates of uniform size (12 cm. diam.) were used and 20 cc. of sterile oatmeal agar poured into each. Four plates were inoculated with each of the three isolates and incubated at different temperatures. In some of the incubators the temperature fluctuated by about 1°C. The radial growth measurements of the colonies were recorded daily and the position after eight days' growth is shown in table II.

TABLE II

Mean radial growth of the three isolates on oatmeal agar at different temperatures

Isolate	Mean radial growth in cms. at different temperatures						
	10°C	15°C	20°C	25°C	30°C	35°C	40°C
3	0.4	3.6	7.1	10.6	11.0	11.0	0
29	1.4	5.0	8.0	11.5	12.0	12.0	0
78	1.5	5.2	8.0	11.5	12.0	12.0	0

The results show that isolate 3 is comparatively slow growing at all the temperatures under test. Isolates 29 and 78, however, do not differ much from one another in their rate of growth. Furthermore, all the three isolates seem to have the same critical temperatures for their growth.

Edgerton and Moreland (1920) found the range of growth for *Colletotrichum falcatum* to be from 13° to 37.5°C, with the optimum at about 27°C. According to Sartoris (1929), the fungus could grow even at 3°C, but the growth was very slight. Tims and Edgerton (1932) reported very slow growth at 10°C, rapid at 27°-34°C, and slow at 37°C. Abbott (1938) observed that *Colletotrichum falcatum* produced some growth at 10°C, but the optimum in his case was from 30° to 32°C, and the upper limit slightly above 37.5°C. He did not get any growth at 40°C. Ramakrishnan (1941) showed that the optimum temperature for the growth of the fungus, that he studied, was about 30°C. He did not get any growth at 10°C, but when the plates were removed to room temperature (28°C) growth commenced and gradually spread all over the plate. The findings reported herein agree mainly with those obtained by Abbott (1938).

EFFECT OF HYDROGEN-ION CONCENTRATION

Experiments were carried out to find whether the three isolates had different H-ion relations regarding their growth. A large quantity of cane-leaf decoction was prepared to which a mixed buffer solution was added and aliquots were adjusted colorimetrically, representing a range of pH from 4.0 to 10.0, at an interval of 1.0. The pH was varied by the addition of increasing amounts of sodium hydroxide or malic acid as described by MacInnes (1922). Four plates were poured from each aliquot for each of three isolates, and inoculated with small inoculum consisting of the young vigorously growing mycelium of the respective isolate. These plates were then incubated at 30°C and the diameter of the colonies was measured at the end of eight days' growth. The results obtained are summarized in table III. No visible effect was observed on the sporulation of the three isolates at any of the pH range tested.

TABLE III

Effect of hydrogen-ion concentration on the growth of the three isolates

Isolate	Average diameter of colonies in cm. at different pH*							
	4.0	5.0	5.7	6.0	7.0	8.0	9.0	10.0
3	2.0	7.5	9.5	7.6	6.8	6.5	5.7	0
29	2.5	7.6	9.5	7.7	6.5	6.1	4.3	0
78	2.8	7.8	9.5	7.8	6.0	5.8	4.2	0

*The normal cane-leaf decoction showed of pH 5.7.

Abbott (1938) got the maximum growth of *Colletotrichum falcatum* between pH 5.0 and 6.0, with the optimum near 5.5. He did not get typical growth

at any of the other ranges. Ramakrishnan (1941) found that the best growth occurred between pH 4.5 and 5.0, but the fungus was capable of growing over a wider range. The results obtained in the present study show that the optimal hydrogen-ion concentration of the culture medium for the three isolates lies between pH 5.0 and 6.0, and that there is not much variation in their rate of growth within this range. The light coloured isolate 78 gives better growth on the acidic range, whereas isolate 3 towards the alkaline side. Isolate 29 shows intermediate behaviour in this respect.

MORPHOLOGICAL STUDIES

Spore measurements were made of 15-day old cultures of the three isolates on oatmeal agar, incubated at 30°C. Two hundred conidia were measured in each case. The conidia were mounted in lactophenol, and all those encountered in the microscopic field, from one side of the slide to the other, were measured. Both length and width were measured in the beginning, but when it became apparent that width was less variable than length, only the length measurements were recorded (Table IV).

TABLE IV

Frequency table for length of 200 spores of isolates 3, 29 and 78 of Colletotrichum falcatum

Isolate	Class values in microns and their frequencies				
	14-18	18-22	22-26	26-30	30-34
3	26	58	116	0	0
29	3	28	129	38	2
78	0	12	84	91	13

It will be seen that isolate 3 has the smallest spores and isolate 78 the largest ; isolate 29 is, on the other hand, intermediate to both. The general means for isolates 3, 29 and 78 are 22.1, 26.6 and 27.1 μ, respectively. There is, however, a greater predominance of larger spores in isolate 78 than in isolate 29.

Went (1893), in his original description of *Colletotrichum falcatum*, gave the general mean of conidial length as 25.0 μ. According to Butler (1906), the range was from 20-30 μ. Abbott (1938) studied 81 isolates of the fungus and found the range to be 16-48 μ, but his general mean was 25.1 μ. Our results show that the range of spore length for the three isolates studied is 14-34 μ and the average general mean 25.2 μ.

PATHOGENECITY

Ten fully grown cut canes of the variety Co. 213 were inoculated by the usual plug method, described earlier in the paper. Table V gives the linear spread of the infection obtained with the three isolates under study. In the control, the red coloration of the cane tissue was confined to the immediate neighbourhood of the point of inoculation. The three isolates inoculated were readily recovered from the respective canes on re-isolation.

TABLE V

Virulence tests with isolates 3, 29 and 78 on the cut canes of Co. 213

Cane No. inoculated	Linear spread of attack in inches		
	Isolate 3	Isolate 29	Isolate 78
1	18.8	38.5	54.0
2	27.5	34.3	50.0
3	18.0	25.0	56.0
4	22.5	32.8	51.0
5	10.3	34.5	50.5
6	29.5	32.0	49.0
7	20.8	39.0	44.0
8	13.0	33.5	44.0
9	25.5	28.0	43.5
10	21.8	36.5	42.5
Mean	20.8 ± 6.0	33.4 ± 4.2	48.5 ± 4.7

Thus, isolate 78 possesses the greatest virulence, isolate 3 the least, and isolate 29 is intermediate. The mean linear spread of attack in the cane for isolates 3, 29 and 78 is 20.8 ± 6.0, 33.4 ± 4.2 and 48.5 ± 4.7 inches, respectively. The differences are statistically significant. It will be further noted that darker the colour of the mycelium of an isolate, less virulent it is.

CONCLUSION

The foregoing data show that there is no marked difference in the growth rate of the three *Colletotrichum falcatum* isolates on different media or under varying temperature and pH conditions.

The three isolates grow best on oatmeal agar and, if a clearer medium is required, cane-leaf decoction can be successfully used. The optimum temperature for their growth is about 30°C, no growth occurs at 40°C and very slight at 10°C. The fungus grows best at pH 5.7. The light coloured isolate 78, however, shows slightly better toleration on the acidic side and isolate 3 towards the alkaline series.

It has also been indicated that darker the colour of the mycelium of an isolate and smaller the size of its spores, less virulent it is. Further work is, however, necessary in this direction with a much wider collection of Indian isolates of *Colletotrichum falcatum*.

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GENERA OF RUSTS*

M. J. Thirumalachar and B.B. Mundkur

Appendix

One of us (M.J.T.) has recently established a new genus, and the genus *Polioma*, which we had treated as a synonym of *Eriosporangium*, has been re-studied by Baxter and Cummins resulting in its being now considered as valid.

This has necessitated the addition of this Appendix to our 'Genera of Rusts'.

February 20, 1951

121. DIDYMOSPORELLA Thirumalachar in *Science & Culture*, **16**, p. 210, 1950 ; *Sydowia*, **5**. p. **29** 1951

Pycnia intraepidermal, applanate. Aecia uredinoid, subepidermal ; aeciospores resembling the urediospores. Uredia subepidermal ; urediospores pedicellate. Telia subepidermal, erumpent, without peridia, producing teliospores in short ephemeral columns ; teliospores 2-celled, hyaline, pedicellate at first, later appearing sessile due to the deliquiscence of pedicels. Teliospores germinating immediately at maturity ; promycelium external, typically four-celled.

TYPE SPECIES : *Didymosporella toddaliae* (Petch) Thirumalachar on *Toddalia aculeata* (Rutaceae)

DISTRIBUTION : India, Ceylon. (Two species)

NOTES : The two species, *Didymosporella toddaliae* (Petch) Thirumalachar and *Didymosporella macrospora* (Mundkur & Thirumalachar) Thirumalachar, had previously been placed in the genus *Didymospora*, on account of the sessile teliospores observed in the mature telial columns (Thirumalachar, 1942). In doing so, the following observations were made : The pycnia are amphigenous and intraepidermal in the *Toddalia* rust, whereas they are subepidermal in *Didymospora* species so far known. The teliospores of *D. toddaliae* are developed on a basal cell, while in *D. macrospora* the basal teliospores are borne on pedicels measuring up to 51μ. The young teliospores, scraped from the telium, retain their pedicels while all the mature teliospores are sessile. A re-study of fresh material by Thirumalachar, in 1949, revealed the stages of the deliquiscence of the septa and their subsequent appearance as sessile teliospores. Since these characters were quite distinct from those of *Didymospora*, these two rusts have been accommodated in a separate genus *Didymosporella*. In the character of the deliquescence of the pedicels, there is a good resemblance with *Gymnosporangium* and

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*The previous parts of the "Genera of Rusts" appeared in INDIAN PHYTOPATHOLOGY **2** : 65-101, 193-244 ; 1949 and **3** : 4 1950

Coleopuccinia. In the latter, the teliospores are embedded in the gelatinous matrix which swells in water and forms a crust on drying. In *Didymosporella*, however, the spore columns are ephemeral and the teliospores separate away. The gelatinous substance, contributed by the deliquiscing pedicels, is very little or none at all.

The recently published genus, *Gywnopuccinia* Ramakrishnan (*Trans.* Brit. Mycel. Soc. 1950) is undoubtedly a synonym of this genus.

Thirumalachar, M.J. (1942) .. .. *Proc. Indian Acad. Sci.* **16** : 165-174.

122. **POLIOMA** Arthur, emend Baxter & Cummins in *Bull. Torrey Bot. Cl.* 1951 (in press)

Pycnia subepidermal, globoid, paraphysate. Aecia subepidermal, caemoid ; aeciospores catenulate. Uredia subepidermal, erumpent ; urediospores pedicellate. Telia subepidermal, erumpent, compact ; teliospores two-celled, sessile, usually clustered, with a single germ-pore in each cell of the spore ; wall hyaline.

TYPE SPECIES : *Polioma nivea* (Holw.) Arth. on *Salvia purpurea* Cav. (Labiatae)

DISTRIBUTION : Mexico, Central & South America. (Four species)

NOTES : Arthur (1907) established the genus *Polioma*, primarily on the basis of life-cycle, to include, microcyclic species of *Puccinia* the teliospores of which had colourless walls and which germinated without a rest period. The teliospores were described as being pedicellate. *Polioma* was till now considered as synonymous with *Eriosporangium* by Dietel, and we have included it as a synonym of *Eriosporangium* (1949). The type of the genus was re-examined by Baxter and Cummins who kindly allowed us to examine their unpublished manuscript, for which we wish to express our thanks. The genus *Polioma* is not a segregation from *Puccinia*, from which it is separated because it has sessile teliospores as against the pedicellate teliospores of the latter. The clustured nature of the teliospores on sporogenous basal cells indicates one of the modes of teliospore development and has no generic significance. The sessile nature of the teliospores justifies the reinstatement of *Polioma* as valid genus.

Arthur, J.C. (1907) .. .. *J. Mycol.* **13**, p. 29.

Thirumalachar, M.J. & Mundkur, B.B. (1949) *Indian Phytopathology* **2**, p. 202,

Book Review

INTRODUCTION TO THE BACTERIA. 1st Edition

Clifton, C. E., Stanford University ; McGraw-Hill Book Company, New York. 528 pages. 1950.

This newcomer to the field of books on bacteriology represents a carefully executed attempt, to combine essential bacteriological facts with emphasis on general, biological and biochemical aspects, in order to assist the student in learning and comprehending the subject matter. Dr. Clifton has succeeded in collecting numerous investigations and presenting the essential information in a most readable, well planned, and inclusive treatise and thus has rendered a distinctive service to the science of bacteriology.

In the body of the book the manner of presentation differs somewhat from the usual one. It gives in lucid form, some of the latest developments in the field of bacteriology, that is commendable. The text is arranged in twenty-four chapters, with the following captions : introduction to the bacteria ; the observation of bacteria ; the structure of bacteria ; the protozoa and algae ; the true fungi ; the bacteria ; the viruses ; energy requirements of bacteria : mechanisms of microbial respiration ; metabolic groups of bacteria ; growth of requirements of bacteria ; the multiplication of bacteria ; death of bacteria ; microbial variations ; classification of the bacteria ; bacteriology of soil ; bacteriology of water ; bacteriology in the air ; the preparation and preservation of food ; industrial microbiology ; infection and resistance ; the serological reactions ; the Enterobacteriaceae ; and microbiology of infectious disease.

As the author himself points out in the preface, the book does not treat all the areas or problems now embraced in general bacteriology, but, instead, "the field of bacteriology is surveyed in such a manner as to introduce the student not only to descriptive bacteriology but also to modern concepts of the *How* and the *Why* of bacterial behavior". The contents of chapters 8, 9, and 10 dealing with bacterial metabolism are particularly very stimulating and thought-provoking both to the student as well as to the teacher. One feels, however, that, in places, the book contains unnecessarily lengthy and speculative considerations. It is regrettable that such a procedure has required the omission of pertinent details on methods of systemetic study, in order to avoid, probably, a volume of too much bulk. A chapter, or two, on this aspect would have been of marked value.

The publication is well illustrated by excellent photographs and there are numerous tables and graphs. The printing and general format are excellent. It should prove useful to the beginners and advanced students, although it would have been much more valuable if it included a more complete bibliography. This reviewer further opines that those teaching an introductory course in general bacteriology give it a serious consideration.

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Published & edited by R. S. Vasudeva for the Indian Phytopathological Society
Pusa Buildings, New Delhi 12
& printed at Caxton Press, New Delhi